

Authors

Steven Fischer and Theodore Sana *Agilent Technologies*

Adding Retention Times to the METLIN Personal Metabolite Database to Improve Compound Identification

A key step in metabolomics research is the identification of metabolites. Searching accurate-mass LC/MS data against metabolite databases is a powerful technique for identifying metabolites. Due to the presence of isobaric masses such as compound isomers and diastereomers, database searching by mass alone is generally insufficient for positive identification. Public databases do not contain retention time information and thus there is no way to resolve the issue of isobaric masses. However, with Agilent Technologies' METLIN Personal metabolite database, empirically derived, method-specific retention times (RTs) can be added to compounds in the database, creating an accurate-mass retention time (AMRT) database. Retention times then provide a second match criterion orthogonal to, and independent of, compound mass. Searching an AMRT database provides a narrower, more focused list of possible metabolite identities. This technical note discusses the addition of retention times to the METLIN Personal metabolite database.

Our measure is your success.

Agilent Technologies



Adding retention times to create an AMRT database

Retention times can be added to the METLIN Personal metabolite database in a manual process of running metabolite standards, selecting the proper database entry for each standard, and updating the retention time in the database. This approach is effective, but can be slow. A faster, easier approach uses the Agilent MassHunter Mass Profiler software to preprocess the data file from the analysis of metabolite standards before database searching. This approach filters out non-standards and simplifies the assignment of standard matches and addition of retention times in the METLIN software. The workflow is outlined in Figure 1.

Analyzing the metabolite standards

The first step in the workflow is to acquire accurate-mass data from metabolite standards. As many standards as is convenient may be analyzed in a single sample; however, each standard must have a unique mass range around it that is free of interfering masses. If two or more metabolite standards have masses within 5 mDa (or 50 ppm, whichever is larger) of each other, they should be analyzed in separate samples.

For the retention times to be useful, the metabolite standards and experiment samples must be analyzed using the exact same chromatographic method. Ideally, a "universal" chromatographic method that produces good results for both positive and negative ion polarities and for both electrospray and APCI should be used. This makes the resulting AMRT database useful for a wider range of compounds. Agilent recommends a water/methanol mobile phase with approximately 5 mM ammonium acetate and 0.1% acetic acid.

Chromatographic methods optimized for a particular ionization polarity, ionization technique, or analyte can be used, but the resulting retention times will only be meaningful for compounds analyzed with that specialized method.



Figure 1. Workflow for batch addition or update of retention times in the METLIN Personal metabolite database.

Once the metabolite standard sample(s) have been analyzed, process the data using the MassHunter Qualitative Analysis software. Use the Molecular Feature Extraction (MFE) algorithm to locate the compounds and save the resulting data file.

Processing the standards data with the

MassHunter Mass Profiler software The data acquired by analyzing the metabolite standards are processed in the Mass Profiler software to generate a Feature Summary table of the standards and retention times. This table is uploaded to the METLIN Personal metabolite database and is used to add or update retention times for the metabolite entries.

- Create a new project in the Mass Profiler software. Specify one group in the project and add the data file(s) (.mhd) created from the metabolite standards (see Figure 2).
- Open the data file from the standards analysis and select Method → Edit Method on the menu bar.
- In the Pre-Analysis Filters tab, choose the Special Masses option Limit to These (Figure 3). Enter or paste the exact neutral masses of the metabolite standards and click OK. Entering the exact neutral masses here allows the Mass Profiler software to filter out all non-standards (for example, chemical noise or impurities) that may have been detected

in the metabolite standards sample. This significantly simplifies subsequent METLIN database searching and retention time assignment or updating.

- Save the method so that it can be applied to the same type of standard mix in the future.
- 5. Export the feature table as a comma separated value file (.csv).

w Project		Method Paramete
inecifu Project Name	Specify the number of groups in the project	Pre-Analysis Filters
Urine Standard	⊙ 1 O 2	Feature position
Group name Experiment		🕑 Use a
Samples Add	leiete	RT 0
Scalar File Name		Mass 🕕
		- Isotope pattern -
		Formula
		Normalize
		Charge state
		O Any O M
		Abundance (pe
		0
		ő

Analysis Filters Alignment & Normalization Result Filters	
Feature position	Special masses
V Lise all the available data	
Min Mau	C Explande (*) Limit to these Tolerance 0.0050 B
HI 0.0 min.	59.0371 74.0368 75.0320 75.0684 76.0160 88.0160 89.0477 92.0473 103.0633 104.1075 105.0426
Mass 00 Da	112.0273 113.0589 115.0633 116.0110 118.0266
	126.0429 130.0266 130.0630 131.0582 131.0695
Isotope pattern	Interest interest interest
Formula O Custom	
	Mass defect
	Peptide like
Formula	Target a non b sope
	defect 0.00 Da + 0.00000 x mass
	Deviation allowed 0.50 Da
Normalized height error	
0.15	C Features of unknown mass
	Included . Excluded . Limited to these
	C manage C Landed C Landed to dieve
	Number of ions
Charge state	0.1
	2 0
O Any O Mult charge required Mult charge forbidden	0 <
Abundance (per file)	Neutral losses
	Tolerance 0.0050 Da
Min relative abundance	Foreigine (1990) Da
 Min absolute abundance 	
Top largest	
45000.0 Count	
	OK Cancel

Figure 3. Filter out non-standards data from the analysis by specifying the exact neutral masses of the metabolite standards.

Creating an accurate-mass retention time (AMRT) METLIN database using an imported mass-RT list

After processing the metabolite standards data in the MassHunter Mass Profiler data, use this procedure to create a copy of the METLIN Personal database and add retention times for the metabolite standards to the database.

- In the METLIN software, select File → New Database. The original METLIN Personal metabolite database is write-protected so a "new" database must be created before retention times can be added.
- 2. Select an existing database from which to begin (Figure 4). Enter a new name and a description, and click Create.
- 3. In the Batch Search tab (Figure 5), choose Optional for Retention times .
- Click File, and in the dialog box that appears, select the Summary Feature table (.csv) for the metabolite standards that was exported from the Mass Profiler software.
- 5. Click Find Metabolites on the toolbar.



Figure 4. Create a new METLIN Personal metabolite database from an existing METLIN database.

Cherr [Load a Ress/RT list from a file] Restrict mes Dig for a file Restrict mes Dig for a file Restrict mes Dig for a file Restrict mes Restrict mes Dig for a file Restrict mes Restrict		
RT tolesance: 0.1 min		
tch Search Results: No hits for Mass: RT:		
Best Name Formula Mass Deha Mass RT Delka RT CAS	METLIN KEGG	н

Figure 5. Upload the standards Feature Summary table (.csv) to the new METLIN database.

6. One standard at a time, highlight the standard mass in the Batch Search list and select the correct metabolite identity in the Batch Search Results (Figure 6). In almost all cases, there will be more than one match due to isobaric compounds in the database, but since the identity of each analyzed standard is already known, it should be easy to select the correct metabolite from the search results.

Note: If a metabolite standard is not present in the database, add the standard in the Edit Metabolites tab. In the Edit Metabolites tab, click the Add New button, enter the appropriate metabolite information, and click Save As New. Return to the Batch Search tab and select the newly added metabolite as the identity of the standard. Once all of the metabolite standards are assigned identities from the database, review the results in the Batch Summary tab (Figure 7). When all of the assignments are correct, you are ready to update the database.

File I	nt METLIN Database	Personal Metabolite D Edit Metabolites View	Atabase - C: Wa Metabolomics Links	issHunter\databases\Metlin-Ur Help	ine.mtl							
Find f	Metabolites le Search	Batch Search	Batch Summ	ary Edit Metabolites								
Mass	ses:	Here DT	10 million	Masses			Molecule	Structure	MOL Text			
File		Mass HI	HIRS OF	O [M+H]+ Neutral	○ [M-H]-				[Hor Fold]			
1 110		377.10310 13	0.04 20	10			-		P			
Clear		140 06770	F.0 11	Mass tolerance:	o ppm O m	/a			но			
		222 11717 1	1.19 10	Retention times	- Radical ion s	earch mode				~		
		114.0692 1(1.10 10	O Ignore	Include I	neutrals						
	-	114.0003 10	1.57 5	(a) Optional	🗖 Inshuda	minna						
		104.04742 16	270 7	Optional		armorns				04		
		104.04742 10	0.72 7	Required	Include	cations	Notes	Geige vol	3 n 108			
	-	204 14050 15	5.EC C	RT tolerance: 0.1 m	in			arongy row	o p. 100			
		102.0025	0.00 6									
Batch	Search	Results: 11 hits for I	Mass: 146.057	78 RT: 5.9								
Be	st	Name		Formula	Mass	Delta Mass (ppm)	RT (min)	Delta RT	CAS	METLIN	KEGG	HMP
Be	st Adipic	Name		Formula C6H10D4	Mass 146.05791	Delta Mass (ppm) 0.88	RT (min)	Delta RT	CAS	METLIN	KEGG	НМР
Be	st Adipic	Name acid >2-hydroxybutyric acid		Formula C6H10D4 C6H10D4	Mass 146.05791 146.05791	Delta Mass (ppm) 0.88 0.88	RT (min)	Delta RT	CAS <u>124-04-9</u> 3142-65-2	METLIN 115 303	KEGG	HMP
Be	st Adipic	Name acid >2-hydroxybutyric acid ic acid		Formula C6H1004 C6H1004 C6H1004	Mass 146.05791 146.05791 146.05791	Delta Mass (ppm) 0.88 0.88 0.88	RT (min)	Delta RT	CAS <u>124-04-9</u> <u>3142-65-2</u> 541-07-1	METLIN 115 303 470	KEGG	HMP
Be	st Adipic - 2-Aceto Mevald] alpha-K	Name acid >2-hydroxybutyric acid lic acid etopantoic acid		Formula C6H1004 C6H1004 C6H1004 C6H1004	Mass 146.05791 146.05791 146.05791 146.05791	Delta Mass (ppm) 0.88 0.88 0.88	RT (min)	Delta RT	CAS 124-04-9 3142-65-2 541-07-1 470-30-4	METLIN 115 303 470 485	KEGG	HMP
Be	st Adipic - 2-Aceto Mevald alpha-K 1 Isosorb	Name acid >2-hydroxybutyric acid lic acid ietopantoic acid ide		Formula C6H1004 C6H1004 C6H1004 C6H1004 C6H1004 C6H1004	Mass 146.05791 146.05791 146.05791 146.05791 146.05791	Delta Mass (ppm) 0.88 0.88 0.88 0.88	RT (min)	Delta RT	CAS 124-04-9 3142-65-2 541-07-1 470-30-4 652-67-5	METLIN 115 303 470 485 863	KEGG	HMP
Be	st Adipic A 2-Aceto Adipic A 2-Aceto Alpha-K Isosorb adipate	Name acid -2-hydroxybutyric acid lic acid iceopantoic acid ide		Formula C6H1004 C6H1004 C6H1004 C6H1004 C6H1004 C6H1004 C6H1004	Mass 146.05791 146.05791 146.05791 146.05791 146.05791 146.05791	Delta Mass (ppm) 0.88 0.88 0.88 0.88 0.88	RT (min)	Delta RT	CAS 124-04-9 3142-65-2 541-07-1 470-30-4 652-67-5 764-65-9	METLIN 115 303 470 485 863 3746	KEGG	нмр
Be	st Adipic - 2-Aceto 2-Aceto Aevald alpha-K Isosorb adipate 3-Meth	Name acid -2-hydroxybutyric acid iceopantoic acid ide Aglutaric acid		Formula C6H1004 C6H1004 C6H1004 C6H1004 C6H1004 C6H1004 C6H1004 C6H1004	Mass 146.05791 146.05791 146.05791 146.05791 146.05791 146.05791 146.05791	Delta Mass (ppm) 0.88 0.88 0.88 0.88 0.88	RT (min)	Delta RT	CAS 124-04-9 3142-65-2 541-07-1 470-30-4 652-67-5 764-65-9 526-51-7	METLIN 115 203 470 485 863 3746 3798	KEGG	HMP
Be	st Adipic 4 2-Aceto 2-Aceto 4 Mevald 3 alpha-K 1 Isosorb 3-Meth 2-Meth 2-Meth	Name acid -2-hydroxybutyric acid ic acid ictopantoic acid ide / /glutaric acid /glutaric acid		Formula C6H1004	Mass 146.05791 146.05791 146.05791 146.05791 146.05791 146.05791 146.05791 146.05791	Delta Mass (ppm) 0.88 0.08 0.08 0.08 0.08 0.08	RT (min)	Delta RT	CAS 124-04-9 3142-65-2 541-07-1 470-30-4 652-67-5 764-65-8 526-51-7	METLIN 115 303 470 485 863 3746 3798 5411	KEGG	HMP
Ber	Adipic a 2-Aceto 4 Adipic a 2-Aceto 4 Adipic a alpha-K 1 Isosorb 3-Meth 2-Meth 2-Meth 4 Adipic a	Name acid -2-tydroxybutyric acid fic acid (actopantoic acid ide t (glutaric acid Aglutaric acid scid		Formula C6H1004	Mass 146.05791 146.05791 146.05791 146.05791 146.05791 146.05791 146.05791 146.05791 146.05791	Delta Mass (ppm) 0.88 0.08 0.08 0.08 0.08 0.08 0.08	RT (min)	Delta RT	CAS 124-04-9 3142-65-2 541-07-1 470-30-4 652-67-5 764-65-9 526-51-7 526-51-7	METLIN 115 303 470 485 863 3746 3798 5411 5437	KEGG	HMP
Be	Adipic a 2 Adipic a 2 Acetr 4 Mevald alpha-K 1 Isosorb 3 Adipate 3 Adipic a 4 Adipic a 4 Adipic a	Name acid 5-2-tydroxybutyric acid fic acid (etopantoic acid ide (yglutaric acid yglutaric acid ethyl glutaric acid		Formula C6H1004 C6H1004	Mass 146.05791 146.05791 146.05791 146.05791 146.05791 146.05791 146.05791 146.05791 146.05791 146.05791 146.05791	Delta Mass (ppm) 0.88 0.88 0.88 0.88 0.88 0.88 0.88 0.8	RT (min)	Delta RT	CAS 124-04-9 3142-65-2 541-07-1 470-30-4 652-67-5 764-65-8 526-51-7 54-65-8	METLIN 115 303 420 485 863 3748 3749 5411 5437 5921	KEGG	HMP HMD800422 HMD800448 HMD800448

Figure 6. Confirm that each standard mass in the batch search list is correctly identified in the batch search results. If necessary, select the correct identity for a standard in the batch search results.

Technical Overview

 The "Apply Retention Time" tab will become active once the number of conflicting hits is set to zero. Once active, click Apply Retention Times in the Batch Summary tab. The database is updated to include the retention times (Figure 8) and is automatically saved.

Searching a custom METLIN personal database using accurate masses and retention times

After an AMRT METLIN database is created, it is easy to incorporate retention times into subsequent searches. When specifying the search parameters in the Batch Search tab, simply select Optional or Required under Retention times. Choosing Optional ranks mass matches higher that also match RT; Required returns only mass matches that also have RT matches. As a general rule, choose Optional for general (non-targeted) searches because not all compounds in the database will have retention times. For targeted searches, Required is useful because it eliminates compounds that are not of interest from the results.



Figure 7. Review the metabolite standard information and confirm that there are no conflicting hits.

Conclusions

Adding retention times to metabolite databases provides a second match criterion orthogonal to, and independent of, compound mass. Searching both accurate mass and retention time will often provide a narrower, more focused list of possible metabolite identities, especially for targeted metabolite searches. Unlike public metabolite databases, retention times can be added to the Agilent METLIN Personal metabolite database. The process of adding retention times to the METLIN database can be significantly simplified and accelerated by preprocessing data from metabolite standards with the Agilent MassHunter Mass Profiler software.

ile Database Edit Metabolites View Metz	ase - C. Massiriumer da	itabases Metlin	-Urine.mtl								
Find Metabolites 🎒 🛃 🗋 📂 📀											
Single Search Batch Search	Batch Summary f	Edit Metabolites	7								
Report comments:			-IC		h	dolecule:	Structure MO	Text			
					ſ	2			0		
Mass list search parameters	IC US D					_			\land		
Mass list file: L:\MassHunter\Metlin\Mass List Files	ASynUrine_Pos.csv					_		Í	Т		
Masses: +/- 10 ppm Neutral	Search: Neutrais								L		
Retention time parameters Best	mass match results								ОН		
RT's: +/- 0.1 min (Optional)	Total hits: 73 / 213 ((34.3%)									
	Conflicting hits: 0 / 73 (.0)	%)				Notes: A	nalgesic, Anti-II	nflammatory, Antip	oyretic, platelet-agg	regation 🔥	
	commonly mon of to (.e.					in	hibitor.				
	90 TO NOT	0.0009-001				1.2					
Apply Retention Times	Single matches: 28 / 213	(13.1%)				A	ctive metabolite	e of Acetylsalicylic	: acid (aspirin)		
Apply Retention Times	Single matches: 28 / 213	(13.1%)]			A	ctive metabolite	e of Acetylsalicylic	: acid (aspirin)	×	
Apply Retention Times	Single matches: 28 / 213	(13.1%) Iches, 213 sub	omitted)				ctive metabolite	e of Acetylsalicylic	: acid (aspirin)	<u> </u>	
Apply Retention Times	Single matches: 28 / 213 tal hits, 28 single mat Formula	(13.1%) I ches, 213 sub Mass Submitted	omitted) Mass	Delta Mass (ppm)	RT Submitted	Ar RT (min)	ctive metabolite Delta RT	e of Acetylsalicylic	acid (aspirin)	KEGG	НМР
Apply Retention Times	Single matches: 28 / 213 tal hits, 28 single mat Formula	(13.1%) tches, 213 sub Mass Submitted 138.03143	omitted) Mass 138.03169	Delta Mass (ppm) 1.91	RT Submitted 15.190	A RT (min) 15.190	Delta RT 0.000	e of Acetylsalicylic CAS <u>69-72-7</u>	acid (aspirin) METLIN <u>616</u>	KEGG	НМР
Apply Retention Times	Single matches: 28 / 213 tal hits, 28 single mat Formula C7H603 C6H1005	(13.1%) tches, 213 sub Mass Submitted 138.03143 162.05253	omitted) Mass 138.03169 162.05282	Delta Mass (ppm) 1.91 1.81	RT Submitted 15.190 12.050	A (min) 15.190 12.050	Delta RT 0.000	CAS 69-72-7 503-49-1	METLIN	KEGG	НМР
Apply Retention Times Apply Retention Times Apply Results: 66 hits (73 to Name Salicylic acid 3-hydroxy-3-methyl-Glutaric acid Isoquinoline N-oxide	Single matches: 28 / 213 tal hits, 28 single mat Formula C7H603 C6H1005 C9H7N0	(13.1%) tches, 213 sub Mass Submitted 138.03143 162.05253 145.05253	omitted) Mass 138.03169 162.05282 145.05276	Delta Mass (ppm) 1.91 1.81 1.61	RT Submitted 15.190 12.050 11.750	A RT (min) 15.190 12.050 11.750	Delta RT 0.000 0.000 0.000	e of Acetylsalicylic CAS <u>59-72-7</u> 503-49-1 1532-72-5	e acid (aspirin) METLIN <u>616</u> <u>3793</u> 4111	KEGG	НМР
Apply Retention Times Apply Retention Times Apply Retention Times Action Summary Results: 66 hits (73 to Name Salicylic acid Shydrowy-3-methyl-Glutaric acid Isoquinoline N-oxide 5.6-dimethyl-IH-benzoimidazole	Single matches: 28 / 213 tal hits, 28 single mat Formula C7H603 C6H1005 C9H7N0 C9H10N2	(13.1%) tches, 213 sub Mass Submitted 138.03143 162.05253 145.05253 145.05253	mitted) Mass 138.03169 162.05282 145.05276 146.08440	Delta Mass (ppm) 1.91 1.81 1.61 1.56	RT Submitted 15.190 12.050 11.750 12.380	A RT (min) 15.190 12.050 11.750 12.380	Delta RT 0.000 0.000 0.000 0.000	e of Acetylsalicylic CAS <u>59-72-7</u> 503-49-1 1532-72-5 582-60-5	e acid (aspirin) METLIN 616 3793 4111 4161	KEGG	HMP
Apply Retention Times atch Summary Results: 66 hits (73 to Name Salicylic acid 3rhydroxy-3-methyl-Glutaric acid Isoquinoline N-oside 5.6-dimethyl-1H-benzoimidazole Glu Val Met	Single matches: 28 / 213 tal hits, 28 single mat Formula C7H603 C6H1005 C9H7N0 C9H10N2 C15H27N306S	(13.1%) tches, 213 sub Mass Submitted 138.03143 162.05253 145.05253 145.05477 377.16160	mitted) Mass 138.03169 162.05282 145.05276 146.08440 377.16206	Delta Mass (ppm) 1.91 1.81 1.61 1.56 1.21	RT Submitted 15.190 12.050 11.750 12.300 14.980	A RT (min) 15.190 12.050 11.750 12.380 14.980	Delta RT 0.000 0.000 0.000 0.000 0.000	e of Acetylsalicylic CAS <u>59-72-7</u> 503-49-1 1532-72-5 582-60-5	e acid (aspirin) METLIN 515 3793 4111 4151 16200	KEGG	HMP
Apply Retention Times atch Summary Results: 66 hits (73 to Name Salicylic acid 3-hydroxy-3-methyl-Glutaric acid Isoquinoline N-oxide 5-cl-methyl-TH-benzoimidazole Glu Val Met Valine	Single matches: 28 / 213 tal hits, 28 single mat Formula C7H603 C6H1005 C9H7N0 C9H7N0 C9H10N2 C15H27N306S C5H11N02	(13.1%) tches, 213 sub Mass Submitted 138.03143 162.05253 145.05253 145.05273 145.08477 377.16160 117.07884	mitted) Mass 138.03169 162.05282 145.05276 146.08440 377.16206 117.07898	Delta Mass (ppm) 1.91 1.81 1.61 1.56 1.21 1.19	RT Submitted 15.190 12.050 11.750 12.340 14.340 1.4-0	A RT (min) 15.190 12.050 11.750 12.380 14.980 1.440	Delta RT 0.000 0.000 0.000 0.000 0.000 0.000 0.000	CAS 59-72-7 503-49-1 1532-72-5 582-60-5 72-18-4	e acid (aspirin) METLIN 515 3733 4111 4151 15200 35	KEGG	HMP
Apply Retention Times atch Summary Results: 66 hits (73 to Name Salicylic acid 3-hydroxy-3-methyl-Giutaric acid Isoquinoline N-oxide 5.6-dimethyl-H-benzoimidazole Giu Val Met Valine Desmethyldeschlorobenzoyl Indomethacin	Single matches: 28 / 213 tal hits, 28 single matt Formula C7H603 C6H1005 C9H7N0 C9H7N0 C9H10N2 C1H27N3065 C5H11N02 C5H11N02 C1H3N03	(13.1%) tches. 213 sub Mass Submitted 138.03143 162.05253 145.05253 145.05253 146.08417 377.16160 117.07884 191.05602	mitted) Mass 138.03169 162.05282 145.05276 146.08440 377.16206 117.07898 191.05824	Delta Mass (ppm) 1.91 1.81 1.61 1.56 1.21 1.19 1.19	RT Submitted 15.190 12.050 11.750 12.360 14.980 1.4-00 11.750	A RT (min) 12.050 11.750 12.380 14.980 1.440 11.750	Delta RT 0.000 0.000 0.000 0.000 0.000 0.000 0.000	CAS <u>59-72-7</u> <u>503-49-1</u> <u>1532-72-5</u> <u>582-50-5</u> <u>72-18-4</u> <u>71382-15-5</u>	METLIN 516 3793 4111 4161 16200 35 323 32	KEGG	HMP
Apply Retention Times atch Summary Results: 66 hits (73 to Name Salicylic acid 3-hydroxy-3-methyl-Glutaric acid Isoquinoline N-oxide 5.6-dmethyl-1H-benzoimidazole Glu Val Met Valine Desmethyldeschlorobenzoyl Indomethacin 5(3.4-Dihydroxy-1,5-cyclohexadien-1-9/)-S-ethylbab	Single matches: 28 / 213 tel hits, 28 single mat Formula C7H6D3 C6H1005 C9H7N0 C9H7N0 C9H7N0 C19H2N306S C5H11N02 C19H3N03 C10H9N03 C12H14N205	(13.1%) tobes, 213 sub Mass Submitted 138.03143 162.05253 145.05253 145.05253 145.05417 377.16160 117.07684 191.05602 266.09002 266.09002	mitted) Mass 138.03169 162.05282 145.05276 146.08440 377.16206 117.07698 191.05824 266.09027	Delta Mass (ppm) 1.91 1.81 1.61 1.56 1.21 1.19 1.17 0.95	BT Submitted 15.190 12.050 11.750 12.300 1.4.90 1.4	A RT (min) 12.050 11.750 12.380 14.980 1.440 11.750 13.920	Delta RT 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000	CAS 59-72-7 503-49-1 1532-72-5 582-60-5 72-18-4 71382-15-5 35507-33-1	e acid (aspirin) METLIN 516 3733 4111 4161 16200 35 828 1818	KEGG KEGG C00183	HMP
Apply Retention Times atch Summary Results: 66 hits (73 to Name Salcylic acid 34ydroxy-3-mettyl-Glutaric acid Isoquinoline N-oxide 5.6-dimettyl-1H-benzoimidazole Glu Val Met Valine Desmettyldeschlorobenzoyl Indomethacin 5(3,4-Dirydroxy-1,5-cyclohexadien-1-yl)-5-ettylbarb Carbayl	Single matches: 28 / 213 tel hits, 28 single mat Formula C7H603 C6H1005 C9H7N0 C9H10N2 C15H27N306S C5H11N02 C10H9N03 C12H14N205 C12H11N02 C12H14N205 C12H11N02	(13.1%) total: total	mitted) Mass 138.03169 162.05282 145.05276 145.08440 377.16206 117.07898 191.05824 266.09027 201.07898	Deka Mass (ppm) 1.91 1.81 1.61 1.56 1.21 1.19 1.17 1.17 0.89 0.89	RT Submitted 1519 12.05 11.75 12.30 14.90 14.90 1.40 11.75 13.35 12.91	A RT (min) 12.050 11.750 12.380 14.380 1.440 1.440 1.1750 13.320 12.910	Delta RT 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000	CAS 59-72-7 503-49-1 1532-72-5 582-50-5 72-18-4 71982-15-5 35507-33-1 6325-2	e ecid (aspirin) METLIN 616 3793 4111 4161 16200 35 828 1818 1818 1495	KEGG KEGG C00183 C00183	HMP
Apply Retention Times	Single matches: 28 / 213 tel hits, 28 single matt Formula C7H603 C6H1005 C9H7N0 C9H10N2 C15H27N306S C5H11N02 C15H27N306S C5H11N02 C10H9N03 C12H14N205 C12H11N02 C12H11N02 C12H11N02 C12H11N02 C6H1004	(13.1%) total: total	Amitte d) Mass 138.03169 162.05282 145.05276 146.08440 377.16206 117.07698 191.05624 266.09027 201.07898 146.05791	Deta Mass (ppm) 1.91 1.81 1.61 1.56 1.21 1.19 1.17 0.95 0.89 0.88	PT Submitted 15190 11.750 12.300 14.900 1.4.40 11.750 13.920 13.920 13.930 5.900	Ar RT (min) 15.190 12.050 11.750 12.380 1.4.980 1.4.40 11.750 13.320 12.320 12.300 5.500	Delta RT 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000	CAS 59-72-7 503-49-1 1532-72-5 582-50-5 72-18-4 71392-15-5 35607-33-1 53-25-2 124-04-9	e ecid (aspirin) METLIN 515 3733 4111 4151 15200 35 628 1818 1818 1495 115	KEGG KEGG C00183 C00183 C00183 C00183	HMP

Figure 8. The AMRT METLIN database is updated to include RT information from the standards Feature Summary table.

About Agilent Technologies

Agilent Technologies is a leading supplier of life science research systems that enable scientists to understand complex biological processes, determine disease mechanisms, and speed drug discovery. Engineered for sensitivity, reproducibility, and workflow productivity, Agilent's life science solutions include instrumentation, microfluidics, software, microarrays, consumables, and services for genomics, proteomics, and metabolomics applications.

Buy online:

www.agilent.com/chem/store

Find an Agilent customer center in your country: www.agilent.com/chem/contactus

U.S. and Canada 1-800-227-9770 agilent_inquiries@agilent.com

Asia Pacific adinquiry_aplsca@agilent.com

Europe

info_agilent@agilent.com

This item is intended for Research Use Only. Not for use in diagnostic procedures. Information, descriptions, and specifications in this publication are subject to change without notice.

Agilent Technologies shall not be liable for errors contained herein or for incidental or consequential damages in connection with the furnishing, performance, or use of this material.

© Agilent Technologies, Inc. 2008

Printed in the U.S.A. May 23, 2008

5989-8546EN

Our measure is your success.

