Application Note

Metabolomics

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Identification of Metabolites in Porcine Serum with Hydrogen Carrier Gas

Using the Agilent 8890/5977C GC/MS, the Agilent HydroInert source, and the Agilent Fiehn GC/MS Metabolomics RTL Library

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Abstract

The goal of metabolomics studies is to comprehensively investigate the biological small molecules (metabolites) of biofluids, tissues, and organisms using mass spectrometry (MS). This application note focuses on the analysis of metabolites from the Agilent Fiehn 2013 GC/MS Metabolomics RTL Library on a single quadrupole GC/MS using hydrogen as the GC carrier gas. Method performance between helium and hydrogen carrier gas was examined with a known mix of fatty acid methyl amines (FAMEs) and several known metabolites in matrix. A comparison of library match scores between helium and hydrogen carrier methods demonstrates a 2% to 3% lower library match score when using hydrogen carrier. Identification of the same FAMEs mix when spiked into porcine serum resulted in a match factor range from 93.18% to 98.64%. Additionally, using Unknowns Analysis, 76 metabolites were identified with match factors ≥90%, 110 metabolites with match factors ≥80%, and 143 metabolites with match factors ≥70% in porcine serum matrix. These high quality spectral matching results indicate the Agilent HydroInert source enables the use of the Agilent Fiehn GC/MS Metabolomics RTL Library, in current form, with hydrogen carrier and without the need to acquire hydrogen-specific spectra of metabolites. In this study, known compounds were identified in solution and matrix with minimal spectral tilting when using hydrogen carrier gas and the Agilent HydroInert source.

Introduction

Metabolomics is the systematic study of biological small molecules, which are called metabolites, and are used in the biological processes of organisms. Most metabolomics studies use mass spectrometry to evaluate the metabolite profiles of biofluids, tissues, or organisms. Often the goal of these studies is to provide deeper insights on the function of different genetic backgrounds, the response of disease states, or stimuli like drug treatment. Gas chromatography (GC) paired with MS is well known for being cost-effective, robust, and suitable for routine metabolomics studies. In many cases, less volatile polar metabolites can be made compatible with GC/MS by using chemical derivatization protocols. Derivatization allows investigators to take advantage of not only the robust chromatography but also comprehensive spectral libraries that aid in metabolite detection and identification. To achieve these goals, the Agilent Fiehn GC/MS Metabolomics RTL Library is intended to be part of a solution for laboratories doing metabolomics research using GC/MS to aid in the identification of commonly found metabolites.[1,2] Some of the key advantages of this library are that it includes retention index values for metabolites to help discriminate isomers but also incorporates highly curated spectra of common metabolite TMS derivatives to aid biologists in the interpretation of their results.

Currently, the use of helium carrier gases for GC/MS is becoming more challenging as the globally finite supply of helium gas becomes more subject to shortages and cost increases. Thus, the use of alternative carrier gas, such as hydrogen, is becoming more common due to its low overall cost and abundance of supply. This application note addresses the current helium supply challenge by focusing on the analysis of metabolites from the Agilent Fiehn GC/MS Metabolomics RTL Library on a single quadrupole GC/MS using hydrogen as the GC carrier gas. When adopting hydrogen for GC/MS analysis, there are several factors to consider. First, hydrogen is a reactive gas, and may potentially cause chemical reactions in the inlet, column, and sometimes the MS EI source, which can change analysis results. To address potential issues in the source of the MS, such as spectral tilting or chemical reactions, a HydroInert source was used. The HydroInert source is a substitute for the extractor source when hydrogen carrier is used. It is constructed with materials that greatly reduce undesirable reactions in the source to maintain spectral fidelity when used with hydrogen. Additional information can be found in the Agilent technical overview of the HydroInert source.[3] Second, for GC/MS applications, hardware changes in the gas chromatograph and mass spectrometer may be required when switching to hydrogen carrier gas. The *Agilent Helium to Hydrogen Carrier Gas Conversion Guide*[4] describes in detail the steps for conversion from helium to hydrogen carrier gas. Lastly, it is recommended that anyone working with flammable or explosive gases take a lab safety course covering proper gas handling and use. Further information on the safe use of hydrogen can be found in the *Agilent Hydrogen Safety Manual*[5] and *Hydrogen Safety for the Agilent 8890 GC System Guide.*[6]

Experimental

Chemicals and reagents

Fatty acid methyl amines (FAMEs), myristic acid d_{27}, pyridine, and MSTFA/1% TMCS were obtained from the Agilent Fiehn GC/MS Metabolomics Standards Kit (part number 400505). Methanol (purity 99.9%) was purchased from Sigma-Aldrich (St. Louis, MO, US).

Instrumentation

Instrument operating parameters are listed in Table 1. Table 2 contains a list of consumable items used for the current application. The column configuration was created by attaching the guard column to the analytical column with an inert ultimate union. This configuration allows for quick replacement of the guard column at minimal cost.

Derivatization of standards

FAMEs/myristic acid d_{27} mix was derivatized following the instructions provided with the standards kit (part number 400505) to create a hydrogen retention index file and to retention time lock the method at 16.680 minutes. Ten microliters of pyridine were added to a vial, then 10 µL of the FAMEs/myristic acid d_{27} mix. Finally, 80 µL of MSTFA/1% TMCS were added to the vial. The vial was capped and vortexed for 30 seconds. Then, the vial was incubated at 37 °C for 30 minutes. After incubation, the vial was run on the GC/MS.

Table 1. GC and MS conditions.

Agilent 8890 GC with Fast Oven, Auto Injector, and Tray	
Injection Volume	1.0 µL
Inlet	EPC split/splitless
Mode	Split
Split Ratio	10:1
Septum Purge Flow Mode	Standard, 3 mL/min
Inlet Temperature	250 °C
Oven	Initial: 60 °C (1 min hold) Ramp 1: 10 °C/min to 325 °C (10 min hold)
Guard Column	Fused Silica Retention Gap 10 m × 0.18 mm, 0.0 µm
Column 1	Agilent J&W DB-5ms, 40 m × 0.18 mm, 0.18 µm
Control Mode	Constant flow, 1.2 mL/min
RTL Compound (Time)	Myristic acid d_{27} (16.680 min)
Agilent 5977C MS	
Source	Agilent HydroInert source
Drawout Lens	9 mm
Transferline Temperature	290 °C
Source Temperature	250 °C
Quadrupole Temperature	150 °C
Mode	SCAN
Scan Range (Speed)	50 to 600 (N = 2)
Delta EMV	188
Solvent Delay	5.9 min
Threshold	150
Trace Ion Detection	On
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Table 2. Agilent consumables and part numbers.

Consumable	Description	Part Number
GC Vials	Amber, microsampling, V-bottom	5184-3554
Vial Caps	Screw, blue, certified, PTFE/silicone/PTFE septa	5182-0723
Injector Syringe	Blue Line autosampler syringe, 10 µL	G4513-80220
Inlet Septum	Advanced Green septum, nonstick, 11 mm	5183-4759
Inlet Liner	Universal Ultra Inert mid-frit inlet liner	5190-5105
Gold Seal	GC inlet gold seal, Ultra Inert	5190-6144
Retention Gap	Deactivated Fused Silica, 10 m, 0.18 mm	160-2615-10
Inlet Nut	Column nut, collared, self-tightening	G3440-81011
Ferrules	15% graphite/85%Vespel, 0.1 to 0.25 mm column	5181-3323
MSD Nut	Column nut, collared, self-tightening	G3440-81013
Column	J&W DB-5ms, 40 m × 0.18 mm, 0.18 µm	121-5542
Ultimate Union	Union, Ultra Inert	G3182-60581
Gold Ferules	CFT Ferrule Flex Gold flexible metal ferrule, gold plated, 0.4 mm id, for 0.1 to 0.25 mm id	G2855-28501
Steel Tubing	Install kit for GCs, stainless steel	19199S
GC/MS Source	HydroInert complete source assembly for 5977	G7078-67930

Derivatization of porcine serum

Fresh porcine blood was obtained from a local source. A 2 mL sample of fresh blood was centrifuged at 14,000 rpm for 10 minutes. Blood plasma supernatant was removed and placed into a separate 2 mL vial. A 100 µL sample of serum was mixed with 900 µL of methanol in a 2 mL centrifuge tube. The mixture was vortexed for 1 minute. The mixture was then centrifuged at 14,000 rpm for 10 minutes. The supernatant was collected in an Agilent V-bottom vial and blown to dryness under a gentle stream of nitrogen at 40 °C. Ten microliters of pyridine were added to the vial, then 20 µL of the FAMEs/myristic acid d_{27} mix. Finally, 80 µL of MSTFA/1% TMCS were added to the vial. The vial was capped and vortexed for 30 seconds. Then, the vial was incubated at 37 °C for 30 minutes. After incubation, the vial was run on the GC/MS.

Results and discussion

GC/MS methodology

The Fiehn Metabolomics methodology[1] was translated from helium to hydrogen using the Agilent GC method translator software. To compare method performance, the FAMEs test mix was retention time locked, then run using the hydrogen methodology presented above. Figure 1 shows the comparison of chromatograms obtained using helium and hydrogen carrier gas. The difference in observed retention times is shown in Table 3. As a reference, 0.1 minutes is equal to 6 seconds, and the greatest retention time difference was observed at C_{30} with a delta of 0.06 minutes. A comparison of library match scores for both the helium and hydrogen methods are presented in Table 4. In some cases, hydrogen method match scores are 2% to 3% lower than helium match scores. The minimized difference in match scores is expected for hydrogen carrier, which demonstrates minimal spectral tilting when using the HydroInert source.[3]

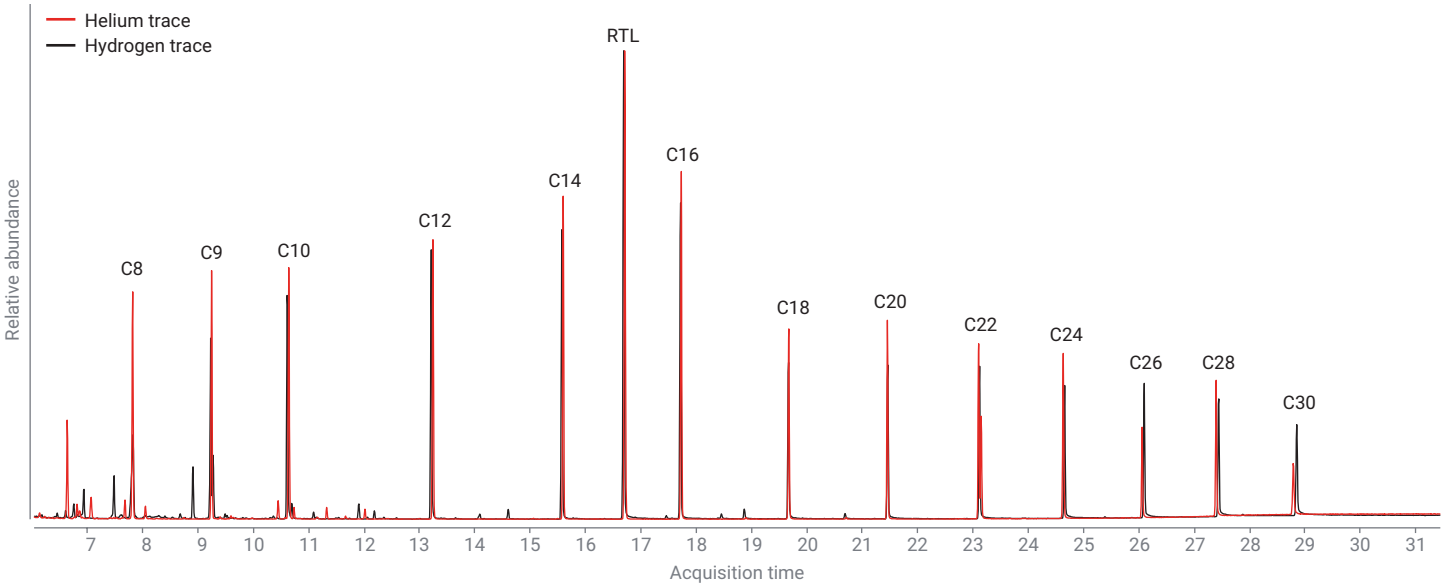

Figure 1. Comparison of the FAMEs calibration standards using helium (red) and hydrogen (black).

Table 3. Retention time of compounds in the FAMEs standard when using helium or hydrogen carrier gas.

Compound Name	Helium RT (min)	Hydrogen RT (min)	RT Delta (min)
[C8] Methyl Caprylate	7.818	7.82	0.006
[C9] Methyl Pelargonate	9.245	9.23	0.016
[C10] Methyl Caprate	10.640	10.61	0.028
[C12] Methyl Laurate	13.243	13.21	0.031
[C14] Methyl Myristate	15.596	15.57	0.024
[RTL] Myristic Acid d_{27}	16.709	16.69	0.020
[C16] Methyl Palmitate	17.728	17.71	0.014
[C18] Methyl Stearate	19.671	19.67	0.005
[C20] Methyl Eicosanoate	21.456	21.46	0.006
[C22] Methyl Docosanoate	23.105	23.12	0.015
[C24] Methyl Linocerate	24.634	24.66	0.026
[C26] Methyl Hexacosanoate	26.060	26.10	0.036
[C28] Methyl Octacosanoate	27.399	27.44	0.041
[C30] Methyl Triacontanoate	28.790	28.85	0.060

Table 4. Comparison of match factors using the Agilent Fiehn GC/MS Metabolomics RTL Library with helium and hydrogen carrier gas. Helium method compounds were library searched using RT matching, but no RI calibration as they followed the retention time lock method. Hydrogen scores were acquired with an RI calibration file and RT matching. RT match criteria was trapezoidal for 10 seconds with a 6 second penalty free space and multiplicative penalization. All library searches were done with pure reverse searching.

Compound Name	Helium Match Factor (%)	Hydrogen Match Factor (%)
[C8] Methyl Caprylate	99.92	99.57
[C9] Methyl Pelargonate	99.93	99.35
[C10] Methyl Caprate	99.89	99.25
[C12] Methyl Laurate	99.93	98.95
[C14] Methyl Myristate	99.88	98.60
[RTL] Myristic Acid d_{27}	99.88	99.26
[C16] Methyl Palmitate	99.90	98.23
[C18] Methyl Stearate	99.89	97.47
[C20] Methyl Eicosanoate	99.88	96.91
[C22] Methyl Docosanoate	99.84	96.56
[C24] Methyl Linocerate	99.87	97.92
[C26] Methyl Hexacosanoate	99.80	97.81
[C28] Methyl Octacosanoate	99.78	96.75
[C30] Methyl Triacontanoate	99.80	97.69

Table 5. The RI calibration table used in Unknowns Analysis. Note, this table must be saved in rich text format (.rtc) with all values on each line separated by commas.

Compound	CAS	RI	RT
C8	111-11-5	800	7.824
C9	173-84-6	900	9.228
C10	110-42-9	1,000	10.612
C12	111-82-0	1,200	13.212
C14	124-10-7	1,400	15.571
C16	112-39-0	1,600	17.714
C18	112-61-8	1,800	19.665
C20	1120-28-1	2,000	21.461
C22	929-77-1	2,200	23.120
C24	2442-49-1	2,400	24.659
C26	5802-82-4	2,600	26.095
C28	55682-92-3	2,800	27.439
C30	629-83-4	3,000	28.850

Conversion of the Fiehn Metabolomics methodology[1] from helium to hydrogen required the use of an alternative column setup. Originally, a 30 m × 0.250 mm, 0.25 µm Agilent J&W DB-5ms 10 m Duraguard column was used. When converting to hydrogen, a reduction in column diameter is required to compensate for the pressure differential of hydrogen. Thus, the guard column normally used in the helium method was exchanged for a 10 m uncoated restriction gap (0.180 mm diameter), connected, via ultimate union, to a 40 m × 0.18 mm, 0.18 µm DB-5ms for use with hydrogen carrier. The use of an ultimate union allows the quick swapping of the guard column, which can be necessary when working with complex biological matrices. By swapping the retention gap, one eliminates the need to trim the column and retention time shifting due to differing column length. Additionally, the inlet liner used in this study was a Universal Ultra Inert mid-frit inlet liner. Fritted inlet liners have previously shown superior performance when working with complex matrices.[7,8] Use of the fritted liner can reduce downtime and frequency of maintenance while ensuring reliable transfer of metabolites to the column.

Porcine serum

A chromatogram of a porcine serum sample with spiked FAMEs standard is presented in Figure 2. All known FAMEs compounds and the RTL compound were identified in the serum matrix. Match factors for known compounds ranged from 93.18% to 98.64% (Table 6). Match scores in actual matrix are expected to be slightly lower due to matrix interferences and differences in derivatization protocols. Seventy-six metabolites were identified with match factors ≥90%, 110 metabolites with match factors ≥80%, and 143 metabolites with match factors ≥70%. Identification of known FAMEs compounds in matrix with match factors above 90% while using hydrogen carrier was considered acceptable.

Table 6 presents all metabolites identified with match factors ≥90%. For comparison, the metabolite retention time (RT) and retention index (RI) values are presented along with the library RT and RI. The use of RI calibration in the current method greatly increased the likelihood of correctly identifying a metabolite. Due to coelution or, in many cases, very small retention time differences, the Agilent Fiehn GC/MS Metabolomics RTL Library relies heavily on retention time matching. The subtle differences in the retention times

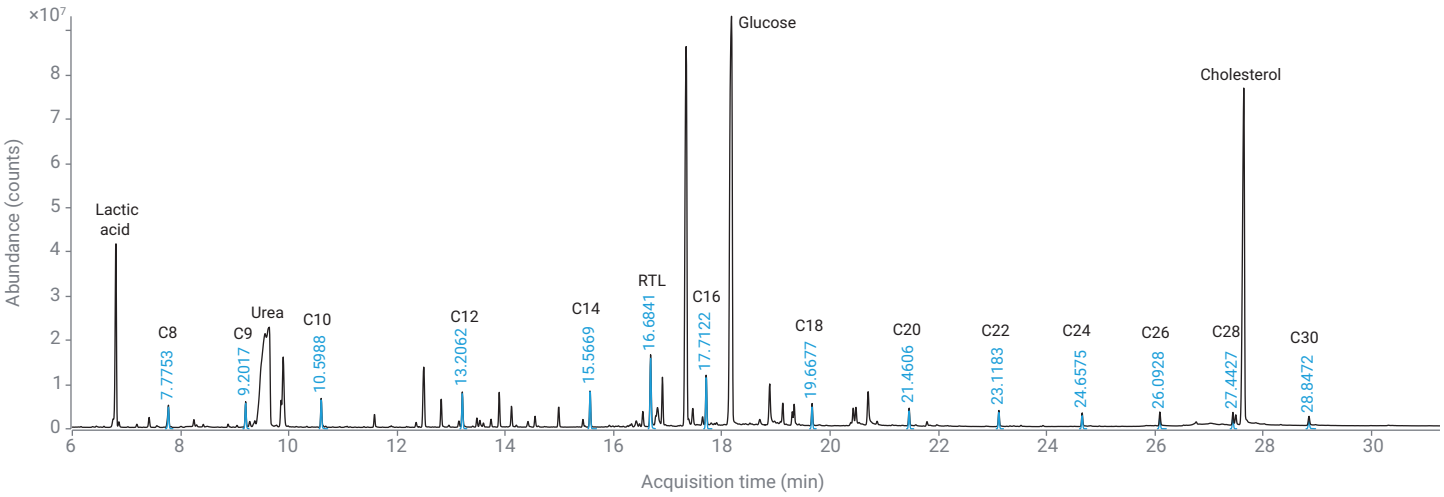

Figure 2. Chromatogram of porcine serum with spiked FAMEs standard. FAMEs compounds are identified in blue.

Table 6. Metabolites detected in porcine serum with match factors ≥90%. Known compounds from the FAMEs mix and the RTL compound are in bold. Metabolite RT and RI values are compared to library RT and RI values. The original RT values from the library when not using RI calibration are present in brackets next to the metabolite name.
* The library RT values presented are modified by the RI calibration file.

Metabolite Name	Metabolite RT (min)	Library RT (min)*	Delta RT (min)	Metabolite RI	Library RI	Delta RI	Match Factor (%)
[8,871] 2-Hydroxypyridine [6.519]	6.485	6.560	0.076	705	710	5	99.48
[107,689] L-(+) Lactic Acid [6.851]	6.804	6.883	0.080	727	733	6	98.04
[757] Glycolic Acid [7.049]	7.027	7.080	0.053	743	747	4	98.77
[11,266] 2-Hydroxybutyric Acid [7.852]	7.753	7.824	0.071	795	800	5	99.37
[C8] Methyl Caprylate [7.812]	7.775	7.824	0.049	797	800	3	98.64
[971] Oxalic Acid [7.883]	7.825	7.852	0.027	800	802	2	98.94
[342] *m*-Cresol [8.03]	8.004	8.007	0.002	813	813	0	94.00
[12,665] 2-Piperidone 1 [8.057]	8.038	8.035	−0.003	815	815	0	98.87
[791] DL-Isoleucine 1 [8.576]	8.530	8.554	0.024	850	852	2	93.88
[867] Malonic Acid 1 [8.919]	8.913	8.905	−0.008	878	877	−1	94.42
[69,362] Beta-Hydroxyisovalerate [9.084]	8.971	9.060	0.088	882	888	6	99.54
[6,287] L-Valine 2 [9.151]	9.043	9.130	0.086	887	893	6	98.25
[C9] Methyl Pelargonate [9.248]	9.202	9.228	0.026	898	900	2	98.09
[8,066] 2-Butyne-1,4-Diol [9.446]	9.373	9.339	−0.034	910	908	−2	90.75
[243] Benzoic Acid [9.594]	9.539	9.491	−0.048	922	919	−3	99.14
[1,176] Urea [9.599]	9.562	9.505	−0.057	924	920	−4	98.32
[379] Caprylic Acid [9.81]	9.750	9.726	−0.024	938	936	−2	98.40
[6,106] L-Leucine 2 [9.945]	9.839	9.865	0.026	944	946	2	97.43
[753] Glycerol [9.941]	9.856	9.865	0.009	945	946	1	98.84
[1,004] Phosphoric Acid [9.966]	9.897	9.892	−0.004	948	948	0	98.33
[791] Dl-Isoleucine 2 [10.225]	10.136	10.169	0.033	966	968	2	97.17
[6,288] L-Threonine 1 [10.224]	10.160	10.169	0.009	967	968	1	98.86
[145,742] L-Proline 2 [10.321]	10.211	10.266	0.055	971	975	4	96.13
[750] Glycine [10.456]	10.334	10.404	0.070	980	985	5	98.89
[1,110] Succinic Acid [10.509]	10.428	10.460	0.031	987	989	2	97.27
[C10] Methyl Caprate [10.647]	10.599	10.612	0.013	999	1,000	1	97.81
[2,969] Capric Acid [12.399]	12.352	12.276	−0.076	1,134	1,128	−6	90.72
[439,766] Citramalic Acid [12.63]	12.490	12.523	0.033	1,144	1,147	3	94.14
[92,824] D-Malic Acid [12.794]	12.701	12.705	0.004	1,161	1,161	0	96.15
[169,019] D-Threitol [12.954]	12.957	12.887	−0.070	1,180	1,175	−5	98.44
[33,032] L-Glutamic Acid 3 (Dehydrated) [13.232]	13.142	13.199	0.057	1,195	1,199	4	98.72
[C12] Methyl Laurate [13.250]	13.206	13.212	0.006	1,200	1,200	0	97.04
[5,460,407] Threonic Acid [13.652]	13.398	13.413	0.015	1,216	1,217	1	97.81
[33,032] L-Glutamic Acid 2 [14.398]	14.320	14.238	−0.082	1,294	1,287	−7	96.54
[7,618] Triethanolamine [14.464]	14.406	14.309	−0.097	1,301	1,293	−8	98.97
[3,893] Lauric Acid [14.789]	14.726	14.675	−0.052	1,328	1,324	−4	98.83
[1,023] Pyrophosphate [14.993]	14.806	14.899	0.092	1,335	1,343	8	98.73

Metabolite Name	Metabolite RT (min)	Library RT (min)*	Delta RT (min)	Metabolite RI	Library RI	Delta RI	Match Factor (%)
[94,154] Arabitol [15.601]	15.326	15.347	0.021	1,379	1,381	2	95.08
[827] Ribitol [15.66]	15.472	15.418	−0.055	1,392	1,387	−5	97.51
[C14] Methyl Myristate [15.597]	15.567	15.571	0.004	1,400	1,400	0	96.25
[754] Glycerol 1-Phosphate [16.056]	15.923	15.871	−0.051	1,433	1,428	−5	98.75
[311] Citric Acid [16.615]	16.540	16.492	−0.048	1,490	1,486	−4	90.49
[RTL] Myristic Acid d_{27} [16.727]	16.684	16.675	−0.009	1,504	1,503	−1	98.00
[219,984] 1,5-Anhydro-D-Sorbitol [16.967]	16.904	16.878	−0.025	1,524	1,522	−2	99.39
[6,508] Quinic Acid [17.076]	16.964	16.996	0.033	1,530	1,533	3	99.33
[5,984] Fructose 2 [17.288]	17.230	17.232	0.002	1,555	1,555	0	95.50
[835] Dehydroascorbic Acid 3 [17.49]	17.463	17.457	−0.006	1,577	1,576	−1	90.22
[5,962] L-Lysine 2 [17.643]	17.536	17.628	0.093	1,583	1,592	9	90.85
[C16] Methyl Palmitate [17.723]	17.712	17.714	0.002	1,600	1,600	0	94.86
[11,850] Galactitol [17.986]	17.826	17.821	−0.005	1,612	1,611	−1	99.64
[5,785] L-Ascorbic Acid [17.939]	17.864	17.773	−0.091	1,615	1,606	−9	91.11
[1,178] Urocanic Acid 2 [18.533]	18.428	18.426	−0.002	1,673	1,673	0	97.99
[128,869] Galactonic Acid 2 [18.504]	18.434	18.397	−0.037	1,674	1,670	−4	90.40
[892] Myo-Inositol [19.354]	19.303	19.324	0.021	1,763	1,765	2	97.61
[1,175] Uric Acid 1 [19.331]	19.339	19.304	−0.035	1,767	1,763	−4	99.71
[5,364,509] Methyl Oleate [19.44]	19.430	19.421	−0.009	1,776	1,775	−1	96.35
[C18] Methyl Stearate [19.663]	19.668	19.665	−0.003	1,800	1,800	0	95.79
[6,305] L-Tryptophan 2 [20.466]	20.435	20.392	−0.042	1,886	1,881	−5	99.02
[637,517] Elaidic Acid [20.508]	20.478	20.437	−0.041	1,891	1,886	−5	96.74
[6,161,490] *trans*-13-Octadecenoic Acid [20.608]	20.535	20.554	0.019	1,897	1,899	2	92.84
[4,114] Xanthotoxin 2 [20.715]	20.703	20.671	−0.033	1,916	1,912	−4	98.45
[C20] Methyl Eicosanoate [21.441]	21.461	21.461	0.000	2,000	2,000	0	94.43
[10,467] Arachidic Acid [22.367]	22.383	22.340	−0.043	2,111	2,106	−5	93.05
[C22] Methyl Docosanoate [23.082]	23.118	23.120	0.002	2,200	2,200	0	94.10
[6,021] Inosine [23.396]	23.394	23.351	−0.043	2,236	2,230	−6	97.26
[60,961] Adenosine [23.825]	23.890	23.813	−0.077	2,300	2,290	−10	99.06
[446,284] Eicosapentaenoic Acid [24.013]	23.933	24.020	0.087	2,306	2,317	11	97.71
[C24] Methyl Linocerate [24.603]	24.658	24.659	0.001	2,400	2,400	0	93.26
[5,283,468] 1 Monoolein [24.747]	24.809	24.716	−0.093	2,421	2,408	−13	90.95
[3,294,434] Digalacturonic Acid 1 [24.822]	24.823	24.795	−0.027	2,423	2,419	−4	90.83
[24,699] 1 Monostearin [24.912]	24.977	24.889	−0.089	2,444	2,432	−12	94.84
[5,280,370] Squalene [25.251]	25.285	25.262	−0.023	2,487	2,484	−3	94.08
[C26] Methyl Hexacosanoate [26.023]	26.093	26.095	0.002	2,600	2,600	0	95.30
[C28] Methyl Octacosanoate [27.349]	27.443	27.439	−0.004	2,801	2,800	−1	93.18
[C30] Methyl Triacontanoate [28.723]	28.847	28.850	0.003	3,000	3,000	0	95.91
[5,321,342] Beta-Sitosterol [28.848]	28.973	28.977	0.004	3,017	3,018	1	94.58

presented in the current hydrogen methodology could result in misidentification without RI values. Numerous examples of potential misidentification are shown in Table 6, where the original library RTs (in brackets) are vastly different from the library RT values which are RI modified. The modified RT values greatly assisted in the positive identification of the 76 compounds that were screened for accuracy. Thus, it is highly recommended to use an RI calibration file when using the Agilent Fiehn GC/MS Metabolomics RTL Library and hydrogen carrier gas.

Use of the HydroInert source greatly reduces the occurrence of redox chemistry in the MS source when using hydrogen carrier.[3] The reduction of chemical reactions in the source leads to conserved spectral fidelity, where spectra generated in hydrogen can be used with a high degree of confidence to identify target metabolites from library spectra generated in helium. To illustrate the conservation of spectra, Figure 3 shows a comparison of [C8] methyl caprylate, [C12] methyl laurate, [RTL] myristic acid d_{27}, [C20] methyl eicosanoate, and [C28] methyl octacosanoate spectra acquired using hydrogen carrier gas, and in porcine serum, to the library spectra, acquired in helium. Match factors for the five standards in Figure 3 are 98.64%, 98.72%, 98.00%, 94.43%, and 93.18%, respectively. A further demonstration of conserved spectral fidelity is presented for five metabolites, glycolic acid, L-valine, urea, D-threitol, and L-glutamic acid 3, identified in porcine serum (Figure 4). The match factors for the five metabolites in Figure 4 are 98.77%, 98.25%, 98.32%, 98.44%, and 98.72%, respectively.

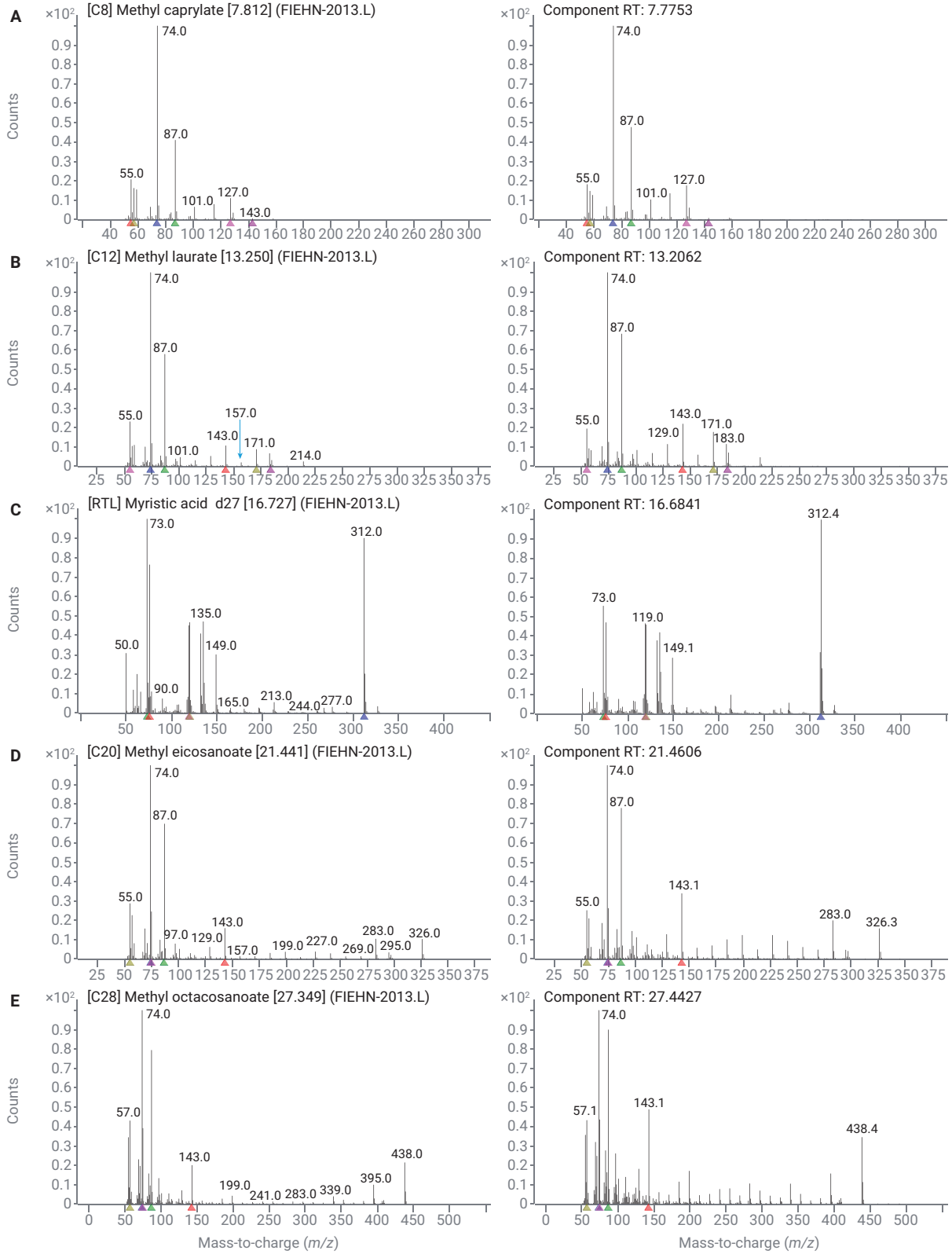

Figure 3. Comparison of Fiehn library spectra (left) to spectra observed in porcine plasma (right), while using hydrogen carrier for (A) [C8] methyl caprylate, (B) [C12] methyl laurate, (C) [RTL] myristic acid d_{27}, (D) [C20] methyl eicosanoate, and (E) [C28] methyl octacosanoate.

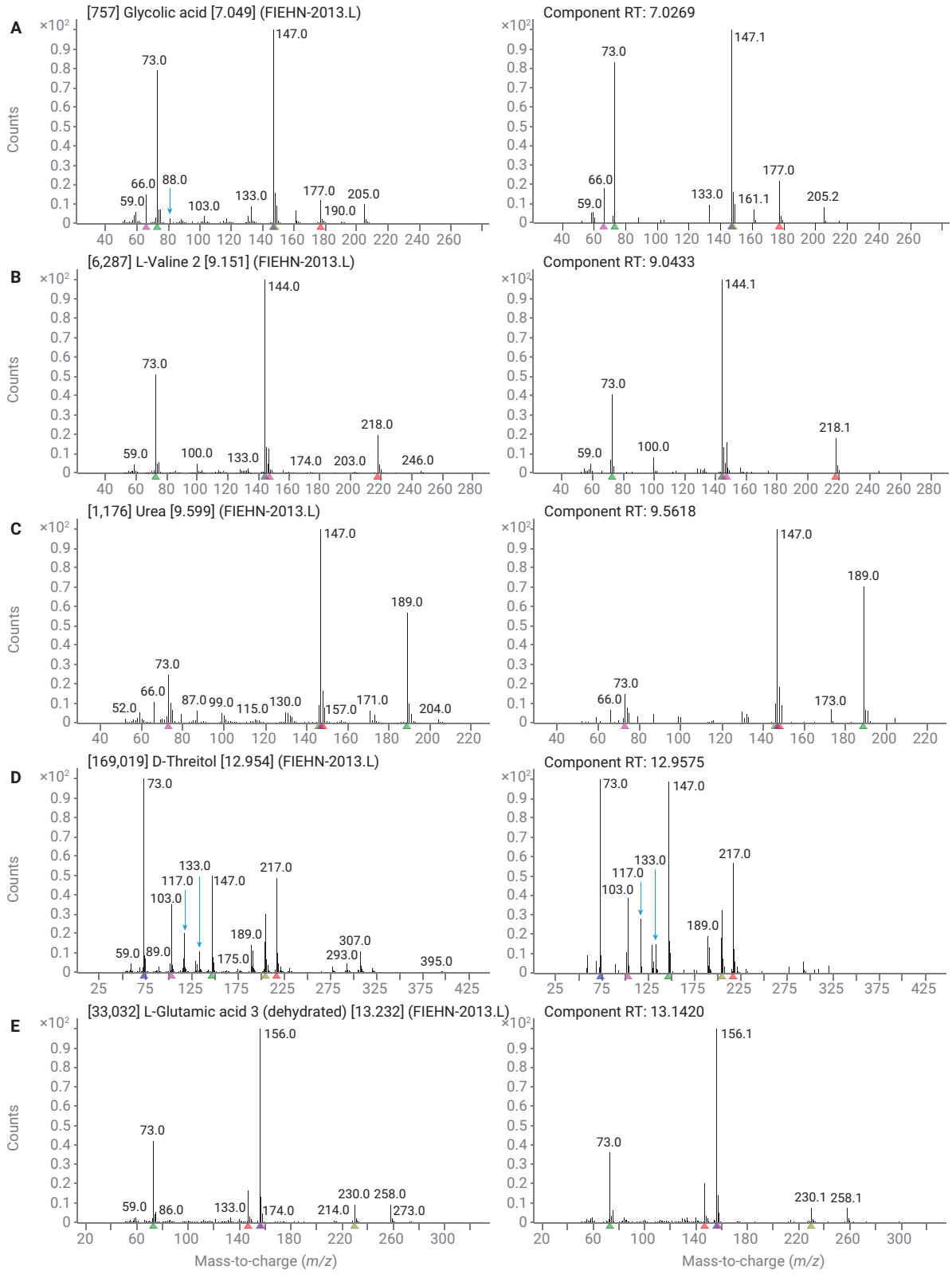

Figure 4. Comparison of Fiehn library spectra (left) to spectra observed in porcine plasma (right), while using hydrogen carrier for (A) glycolic acid, (B) L-valine, (C) urea, (D) D-threitol, and (E) L-glutamic acid 3.

Conclusion

The methodology presented within this application demonstrate that the use of hydrogen carrier gas with the Agilent Fiehn GC/MS Metabolomics RTL Library is possible:

- Known compounds are identified with >90% match factors in solution and in serum matrix.
- Spectral fidelity of analytes is conserved, and spectral tilting is minimized using HydroInert.[3]
- The Agilent Fiehn GC/MS Metabolomics RTL Library, acquired in helium, is suitable for use with hydrogen carrier gas when paired with the Agilent HydroInert Source.

References

1. Agilent G1676AA Fiehn GC/MS Metabolomics RTL Library, *Agilent Technologies technical overview*, publication number G1676-90001, **2013**.
2. Metabolite Identification in Blood Plasma Using GC/MS and the Agilent Fiehn GC/MS Metabolomics RTL Library, *Agilent Technologies application note*, publication number 5990-3638EN, **2009**.
3. Agilent Inert Plus GC/MS System with HydroInert Source, *Agilent Technologies technical overview*, publication number 5994-4889EN, **2022**.
4. Agilent EI GC/MS Instrument Helium to Hydrogen Carrier Gas Conversion, *Agilent Technologies user guide*, publication number 5994-2312EN, **2022**.
5. Agilent GC/MS Hydrogen Safety, *Agilent Technologies user guide*, manual part number G7006-90053, **2022**.
6. Hydrogen Safety for the Agilent 8890 GC System, *Agilent Technologies technical overview*, publication number 5994-5413EN, **2022**.
7. Joseph, S. *et al.* Impact of GC Liners on Lab Productivity While Analyzing Complex Matrices, *Agilent Technologies application note*, publication number 5994-5546EN, **2022**.
8. Haddad, S. P.; Quimby, B. D.; Andrianova, A. A. GC/MS/MS Analysis of PAHs with Hydrogen Carrier Gas, *Agilent Technologies application note*, publication number 5994-5776EN, **2023**.

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