Comparison of Two Beer Samples by GCxGC-TOFMS Utilizing the COMPARE Feature

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Key Words: GCxGC, TOFMS, Compare, Twister SBSE

1. Introduction

The "COMPARE" feature, available in the Data Processing Method of ChromaTOF®, is a single point calibration curve generated from a "Reference" sample, used to compare the Reference sample against a target sample. In this application note, the COMPARE feature will be used to compare two different samples of beer analyzed by SBSE-GCxGC-TOFMS. The objective is to identify variations in twenty targeted compounds between the two samples. The Reference sample is of beer stored cold. The other sample is of the same beer that had been stored at an elevated temperature. For the remainder of this work, the sample from which the reference standard is made will be referred to as the "Reference" and the sample being compared to the reference standard will be called the "Sample".

2. Instruments and Methods

In this study, measurements were made with a LECO Pegasus® 4D GCxGC-TOFMS system. This system consists of an Agilent 6890 gas chromatograph equipped with a LECO dual-jet thermal modulator between the primary and secondary columns and a LECO Pegasus IV Time-of-Flight Mass Spectrometer (TOFMS) as a detector. For this study, the primary analytical column was a GERSTEL-MACH LTM 10.0 m x 0.18 mm ID x 0.20 µm df Rtx-5. The secondary column was a 1.00 m x 0.10 mm ID x 0.10 µm df DB-17ms and was housed in the GC oven. The temperature program for the LTM started at 30°C held for 2 minutes then ramped at 4°C/minute to 230°C and then held for 10 minutes. The column temperature offset for this study was +20°C. The modulator temperature offset for this study was +25°C. Helium was used as the carrier gas at a corrected constant flow of 1.5 mL/minute. The transfer line to the TOFMS consisted of the last 20 cm of the analytical column and was kept at 280°C.

Aliquots of 10 mL were prepared from each sample. The aliquots were placed in 10 mL headspace vials, each containing a 10 mm x 0.5 mm GERSTEL PDMS stir bar and extracted on a stir plate at 900 rpm and 25°C for 120 minutes. Upon completion of the extraction, the stir bar was removed, rinsed with DI water and placed in a GERSTEL Twister Desorption Unit (TDU) tube for analysis. The tube containing the stir bar is loaded into the TDU and the GERSTEL Cooled Inlet System (CIS 4) is cooled. The TDU is then heated, and the analytes are desorbed from the stir bar. The analytes are then trapped in the cooled CIS 4. The CIS 4 is then heated to desorb the analytes onto the GC column.

The TDU was operated in splitless mode. Its initial temperature was 20°C and was held at this temperature for an equilibration time of 30 seconds. It was then heated to 250°C at a rate of 700°C/minute and held at this temperature for 120 seconds. The CIS 4 was cooled to a temperature of -130°C. After a 6 second delay, it was heated to 260°C at a rate of 10°C/second and held for 120 seconds.

3. Results

The cold beer sample was data processed with a Signal-to-Noise ratio (S/N) cut-off of ≥50 and a required spectral similarity against the NIST 05 library of ≥700. A "Reference" was then created and the sample was added as the standard for the Reference. The list of compounds in the Peak Table was reduced to include only the twenty targeted components.

1. N-Nitrosodimethylamine
2. 2-Hexanone
3. Methane sulfonic anhydride
4. Dimethyl trisulfide
5. 1-Octanol
6. Decanal
7. Azulene
8. 4-Benzyloxybenzonitrile
9. Octanoic acid, ethyl ester
10. 1,2,3,4-tetrahydro-1,1,6-trimethyl-naphthalene
11. Isopentyl hexanoate
12. Acetic acid, 2-phenylethyl ester
13. Benzenecarboxylic acid
14. Benzenepropanoic acid, ethyl ester
15. Citronellyl acetate
16. 3-methyl tridecane
17. 3-phenyl-2-propenoic acid, ethyl ester
18. 5-butyldihydro-2(3H)-furanone
19. d-Cadinene
20. Hexadecane

The user-specified criteria that must be set in the Reference are as follows.

"*R.T. Deviation(s)*"*(±)*
the allowable retention time deviation in the 2nd dimension for a Match

"*1st Dimension Retention Deviation*"*(±)*
the allowable retention time deviation in the 1st dimension for a Match (use multiples of the Modulation Period)

"*Match Threshold*"
the minimum spectral similarity for a Match

"*S/N Threshold*"
the minimum S/N for a Match (NOTE: This S/N uses the Quant S/N obtained from the integrated peak, which is different than the Qualitative S/N used by Peak Find in the Data Processing Method) and "Tolerance", which is the allowable variation in Concentration (area in this case, the units are user defined) to be considered a Match.

In this Reference, the following criteria were used.

R.T. Deviation(s):	± 0.2
1st Dimension Retention Deviation(s):	±12
Match Threshold:	700
S/N Threshold:	5
Tolerance (area):	±10%

The next step is to "Calculate Standard" for the Reference. This generates the single point calibration curve that will be used in the COMPARE process. Prior to calculation, the values in the Reference will be green. During calculation of the standard, the values will be red. Upon completion of the calculation of the standard, the values will be black. Figure 1 shows an example of a Reference Table that has uncalculated analytes (A), is calculating analytes (B), and has all analytes calculated and is ready for use (C). Criteria for individual entries in the Reference may be altered, but then must be recalculated by utilizing "Calculate Analyte". The color of the text in the entry will indicate the status of each entry in the Reference. All entries on the Reference must be black for the Reference to perform properly. The calculation of the Reference standard generates a single-point calibration curve. After calculating the Reference, it is necessary to check the log for any errors generated during the calculation of the Reference. The Sample is then automatically compared to this calibration curve, generated from the Reference, in the "COMPARE" process. It should be noted that this is a targeted analysis for peaks present in the Reference.

The next step is to create a Data Processing Method identical to the one used to originally process the sample being used as the reference, except for the addition of the "Compare" box being checked at the top of the DP Method and then the Reference just created is added to the box titled "Add the references to use for comparisons to the list below:"(see Figure 2). The Sample is then data processed using the above DP Method. In the resulting Peak Table, make sure that the "Type" column is visible as this is where the COMPARE-specific information is returned. A section of the COMPARE Peak Table is in Table 1.

There are four different "Types" that can be returned in the Type column in the Peak Table of the sample being compared to the Reference. They are, as follows: Match, Out of Tolerance, Unknown, and Not Found. A "Match" indicates that the peak in the sample matches all of the criteria (R.T.'s, similarity, concentration and tolerance) of the peak in the Reference. A return of "Out of Tolerance" means that the peak in the Sample matches all of the criteria of the peak in the Reference, except that its concentration varies from that of the peak in the Reference by more than the set tolerance (in this case, more than ±10 % area). A return of "Unknown" means that there is no matching peak in the Reference for that peak in the Sample. A return of "Not Found" means that there a peak in the Reference has not been found in the Sample. A return of "Unknown" should not occur for a targeted peak as the "COMPARE" feature is a targeted comparison. In order to return "Unknown" for a peak, that peak must be absent in the Reference and present in the sample. Peaks in the compared Sample that are not included in the Compare Reference will return a type of "Unknown".

A Peak Table for the heat-abused beer being compared to the cold–stored beer is included as Table 1. It has been filtered to only include the 20 targeted compounds. The contents of the Peak Table can be filtered by right-clicking in the Peak Table, selecting "Properties" and then selecting the "Filters" tab. A check box is available for each of the different "Types". Only those types that are checked will be displayed in the Peak Table. Of the 20 targeted compounds, there were 2 Not Founds, 17 Out of Tolerances and 1 Match.

An example for each "Type" is included with its associated mass spectra in Figure 3. In each, the top spectrum is the Peak True deconvoluted spectrum associated with the peak in the Sample being compared to the Reference. It is identified by a red arrow and its trace in the chromatogram is red. In the case of the "Not Found", (C), the Peak True spectrum is replaced by the caliper spectrum taken at the time where the targeted peak marker was placed in the Reference spectrum. This is necessary because a Peak True deconvoluted spectrum is only available where a peak marker has been assigned. Since a corresponding peak was not identified in the sample, there is no associated Peak True spectrum available. The bottom spectrum is the Reference spectrum associated with the peak in the Reference which the sample is being compared to. It is identified by a blue arrow and its trace in the chromatogram is blue.

4. Conclusion

The "COMPARE" feature available in ChromaTOF is a useful tool for tracking changes in targeted compounds between samples by use of a "single-point calibration curve" for each targeted compound. Establishing the Reference matching criteria and confirming an error-free standard calculation is critical to COMPARE performance. Using Peak Table filtering, allows for improved data management of targeted compounds in complex samples, as well as quality control.

(A) Analytes have not been calculated

- "C2_1 Beer sn50 spcal 10%"

#	Name	Masses	Quantitate	Absolute R.T. (s)	R.T. Deviation (s)	1st Dimension Retention Deviation	Match Threshold	S/N Threshold	Area	Height	Concentration	Units	Tolerance	Type
1	N-Nitrosodimethylamine:2	86	Area	222 , 1.975	0.2	12	700	5.0000	34141	1690.7	100.00	%	10.000	Analyte
2	2-Hexanone	58	Area	228 , 1.555	0.2	12	700	5.0000	98757	4738.2	100.00	%	10.000	Analyte
3	Methanesulfonic anhydride:4	79	Area	276 , 0.805	0.2	12	700	5.0000	221431	27801	100.00	%	10.000	Analyte
4	* Dimethyl trisulfide	126	Area	516 , 2.875	0.2	12	700	5.0000	60168	2689.2	100.00	%	10.000	Analyte
5	1-Octanol:2	84	Area	822 , 1.755	0.2	12	700	5.0000	85497	5419.7	100.00	%	10.000	Analyte
6	Decanal	82	Area	954 , 2.010	0.2	12	700	5.0000	35952	1701.2	100.00	%	10.000	Analyte
7	Azulene	128	Area	960 , 3.385	0.2	12	700	5.0000	179193	7242.9	100.00	%	10.000	Analyte
8	4-Benzyloxybenzonitrile	120	Area	990 , 2.475	0.2	12	700	5.0000	111038	2598.4	100.00	%	10.000	Analyte
9	Octanoic acid, ethyl ester:2	88	Area	1014 , 2.025	0.2	12	700	5.0000	5652497	221504	100.00	%	10.000	Analyte
10	* Naphthalene, 1,2,3,4-tetrahydro-1,1,6-t	159	Area	1116 , 2.265	0.2	12	700	5.0000	13831	744.22	100.00	%	10.000	Analyte
11	Isopentyl hexanoate:3	70	Area	1140 , 1.905	0.2	12	700	5.0000	612370	33719	100.00	%	10.000	Analyte
12	Acetic acid, 2-phenylethyl ester	104	Area	1140 , 3.105	0.2	12	700	5.0000	6392540	222947	100.00	%	10.000	Analyte
13	Benzenecarboxylic acid:4	105	Area	1308 , 1.745	0.2	12	700	5.0000	283307	16029	100.00	%	10.000	Analyte
14	Benzenepropanoic acid, ethyl ester	104	Area	1350 , 2.825	0.2	12	700	5.0000	2233567	89364	100.00	%	10.000	Analyte
15	* Citronellyl acetate	94	Area	1362 , 2.010	0.2	12	700	5.0000	3956.6	212.95	100.00	%	10.000	Analyte
16	Tridecane, 3-methyl-	71	Area	1530 , 1.465	0.2	12	700	5.0000	31452	1920.1	100.00	%	10.000	Analyte
17	2-Propenoic acid, 3-phenyl-, ethyl ester	129	Area	1566 , 2.985	0.2	12	700	5.0000	22558	754.96	100.00	%	10.000	Analyte
18	2(3H)-Furanone, 5-butyldihydro-:3	85	Area	1596 , 2.865	0.2	12	700	5.0000	1086218	39330	100.00	%	10.000	Analyte
19	* á-Cadinene	204	Area	1644 , 2.245	0.2	12	700	5.0000	3384.7	210.77	100.00	%	10.000	Analyte
20*	Hexadecane:3	57	Area	1782 , 1.510	0.2	12	700	5.0000	568999	22847	100.00	%	10.000	Analyte

(B) Analytes being calculated

- "C2_1 Beer sn50 spcal 10%"

#	Name	Masses	Quantitate	Absolute R.T. (s)	R.T. Deviation (s)	1st Dimension Retent	Match Threshold	S/N Thres	Area	Height	Concentra	Units	Tolerance	Type
1	N-Nitrosodimethylamine:2	86	Area	222 , 1.975	0.2	12	700	5.0000	34141	1690.7	100.00	%	10.000	Analyte
2	2-Hexanone	58	Area	228 , 1.555	0.2	12	700	5.0000	98757	4738.2	100.00	%	10.000	Analyte
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7	Azulene	128	Area	960 , 3.385	0.2	12	700	5.0000			100.00	%	10.000	Analyte
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9	Octanoic acid, ethyl ester:2	88	Area	1014 , 2.025	0.2	12	700	5.0000			100.00	%	10.000	Analyte
10	* Naphthalene, 1,2,3,4-tetrahydro-1,1,6-t	159	Area	1116 , 2.265	0.2	12	700	5.0000	13831	744.22	100.00	%	10.000	Analyte
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20*	Hexadecane:3	57	Area	1782 , 1.510	0.2	12	700	5.0000	568999	22847	100.00	%	10.000	Analyte

(C) All analytes calculated and ready for use

- "C2_1 Beer sn50 spcal 10%"

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15	* Citronellyl acetate	94	Area	1362 , 2.010	0.2	12	700	5.0000	3956.6	212.95	100.00	%	10.000	Analyte
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20*	Hexadecane:3	57	Area	1782 , 1.510	0.2	12	700	5.0000	568999	22847	100.00	%	10.000	Analyte

Figure 1. **An example of a Reference Table that (A) contains uncalculated analytes, (B) is calculating analytes and (C) has all analytes calculated and are ready for use.**

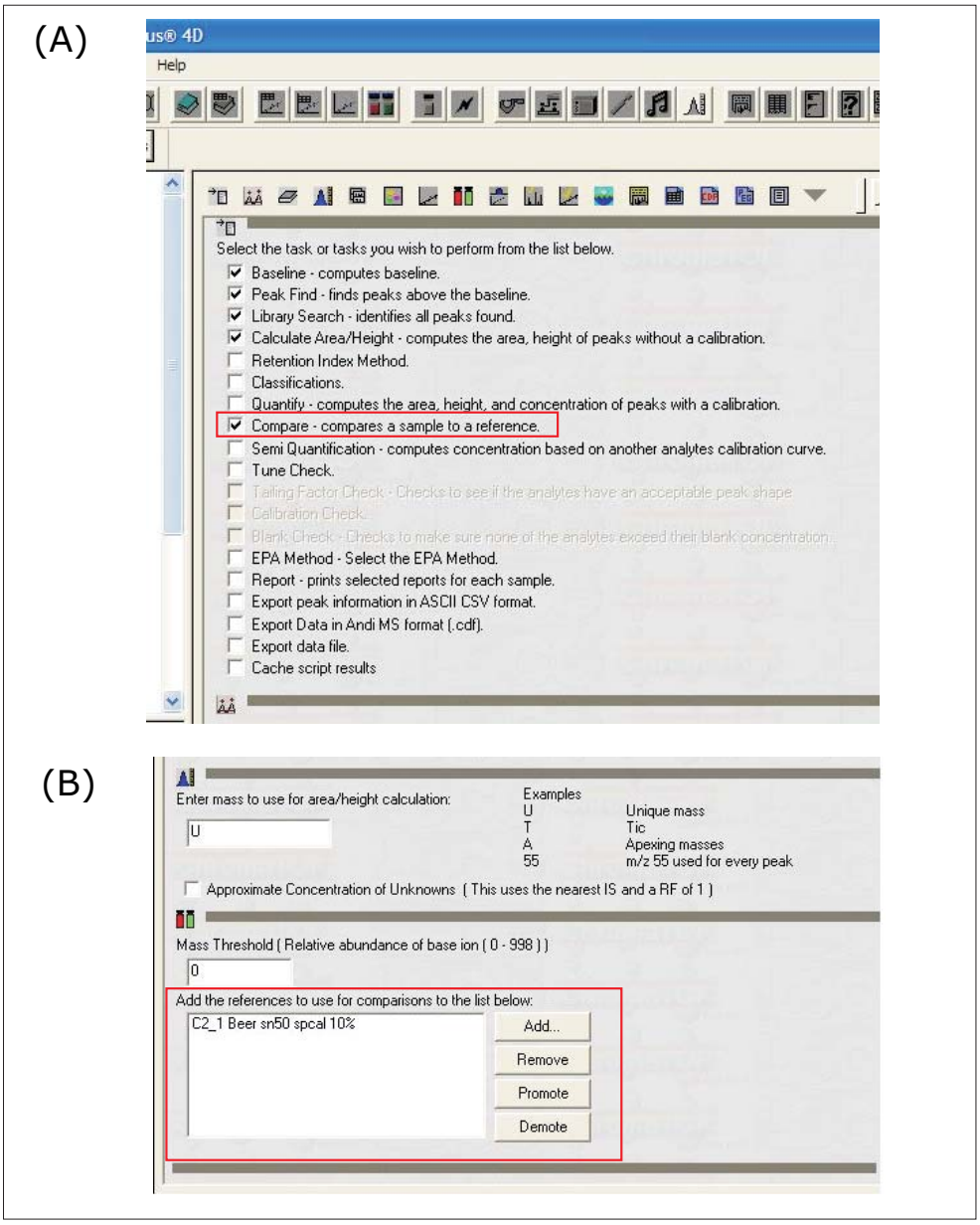

Figure 2. The Data Processing tasks selected for a DP Method using COMPARE are shown in (A). The area at the end of the DP Method where the selected Reference is added is shown in (B). The value for the Mass Threshold in the Reference should match that used in the DP Method originally used to process the sample used in the Reference.

***Table1*. A filtered Peak Table for the heat-abused beer being compared to the cold–stored beer Reference.**

Peak #	Name	R.T. (s)	Type	Unique Mass	Sample Concentration	Match	Quant Masses	Area	% Change
324	N-Nitrosodimethylamine:2	210 , 2.020	Out of Tolerance	86	334.86	796	86	114322	235%
349	2-Hexanone	222 , 1.555	Out of Tolerance	58	34.91	755	58	34474	-65%
831	Dimethyl trisulfide	516 , 2.890	Out of Tolerance	126	154.57	891	126	93003	55%
1763	Decanal	960 , 1.995	Out of Tolerance	82	55.01	725	82	19777	-45%
1771	Azulene	960 , 3.385	Out of Tolerance	128	143.9	852	128	257851	44%
1856	4-Benzyloxybenzonitrile	1002 , 2.460	Out of Tolerance	71	42.63	819	120	47337	-57%
1878	Octanoic acid, ethyl ester:2	1014 , 2.010	Out of Tolerance	88	51.48	972	88	2909696	-49%
2158	Naphthalene, 1,2,3,4-tetrahydro-1,1,6-trimethyl-	1116 , 2.265	Out of Tolerance	159	562.35	853	159	77777	462%
2192	Isopentyl hexanoate:3	1134 , 1.930	Out of Tolerance	70	184.74	922	70	1131314	85%
2239	Acetic acid, 2-phenylethyl ester	1146 , 3.125	Out of Tolerance	91	239.71	973	104	15323690	140%
2785	Benzenecarboxylic acid:4	1320 , 1.700	Out of Tolerance	105	1.09	819	105	3095.7	-99%
2883	Benzenepropanoic acid, ethyl ester	1350 , 2.815	Match	104	98.77	945	104	2205991	-1%
2921	Citronellyl acetate	1368 , 1.950	Out of Tolerance	82	5.95	747	94	235.24	-94%
3340	Tridecane, 3-methyl-	1518 , 1.460	Out of Tolerance	71	116.82	803	71	36742	17%
3488	2-Propenoic acid, 3-phenyl-, ethyl ester	1566 , 3.000	Out of Tolerance	131	224.36	948	129	50610	124%
3534	2(3H)-Furanone, 5-butyldihydro-:3	1584 , 2.960	Out of Tolerance	85	123.35	860	85	1339799	23%
3769	δ-Cadinene	1650 , 2.255	Out of Tolerance	134	42.29	714	204	1431.3	-58%
4240	Hexadecane:3	1782 , 1.515	Out of Tolerance	71	122.51	921	57	697096	23%
	1-Octanol:2/C2_1 Beer sn50 spcal		Not Found			700	84		
	Methanesulfonic anhydride:4/C2_1 Beer sn50 spcal		Not Found			700	79		

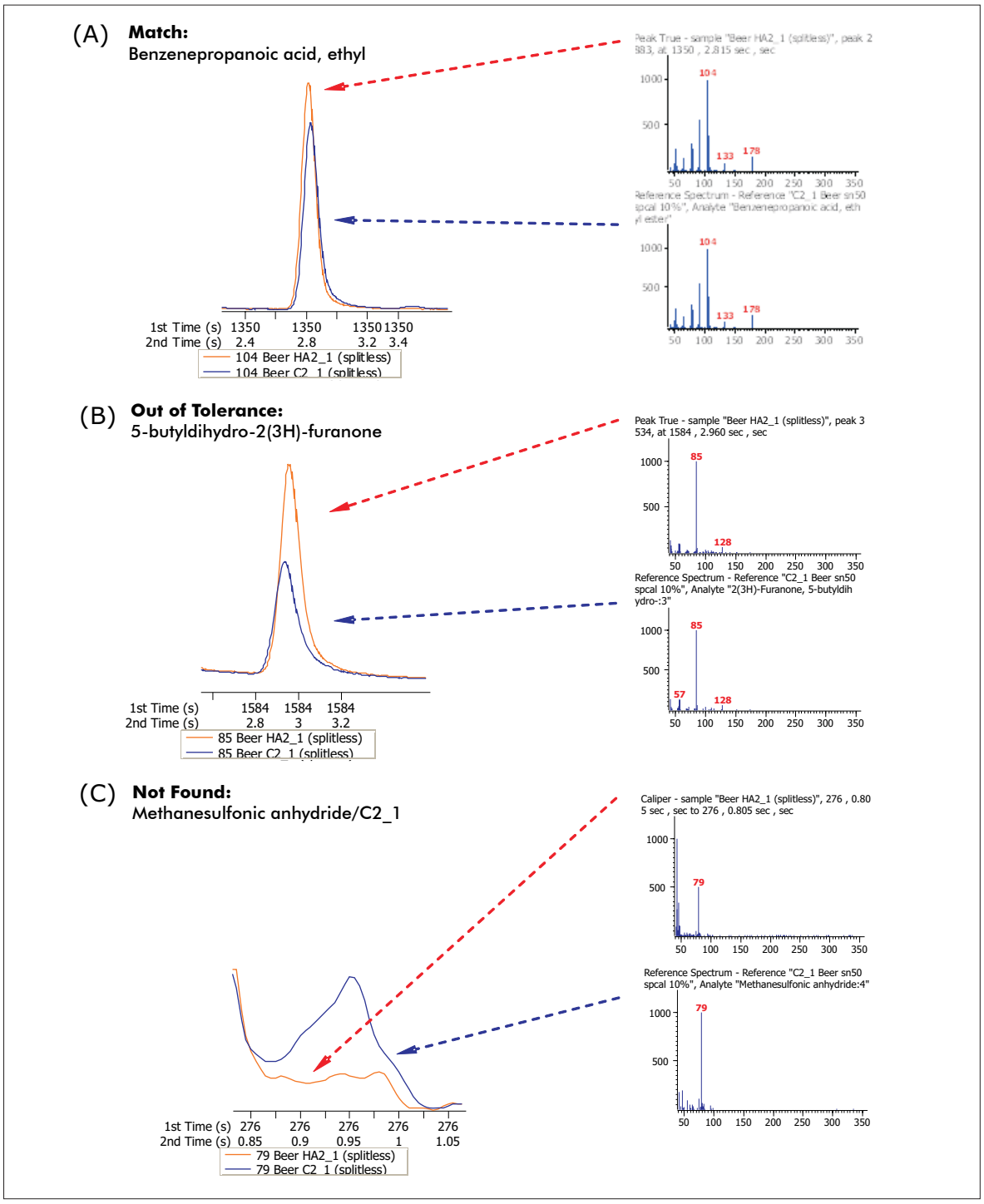

Figure 3.: Examples of chromatograms and spectra for an: (A) Match, (B) Out of Tolerance and (C) Not Found. Red lines indicate association with the Sample and blue lines, the Reference.

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