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ORIGINAL ARTICLE

The Simulacrum System as a Construct for Mass Spectrometry of Triacylglycerols and Others

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Abstract A construct called a simulacrum is defined that provides all possible solutions to a sum of two mass spectral abundances, based on values (abundances) or ratios of those values. The defined construct is applied to atmospheric pressure chemical ionization mass spectrometry (MS) of triacylglycerols (TAG). A simulacrum has precisely defined components, specifically a simulacrum sum, four Possibilities to Observe, two Cases, and eight solutions. A simulacrum with no restrictions is the First General Form of a Simulacrum. When one value is specified to be 1 (as in MS), the construct is called a Unit Simulacrum, also called the First Specified Form of a Simulacrum. When one value is 1 and no value can be greater than 1 (the two specifications dictated by mass spectrometry), the construct is called the Second Specified Form of a Simulacrum, or the Mass Spectrometry Simulacrum. Simulacra are used with three Critical Ratios calculated from raw abundances in mass spectra of TAG to provide structural information about the degree of unsaturation in TAG, the identity and quantity of regioisomers, and other structural characteristics. Three-level-deep nested simulacrum solutions yield the recently reported Updated Bottom Up Solution, from which the protonated molecule, $[MH]^+$, and all diacylglycerol-like fragments, $[DAG]^+$, of TAG can be reproduced from the Critical Ratios. Thus, the simulacrum solutions constitute a reduced data set in which more information is provided in fewer values than raw abundances, such that the Critical Ratios constitute a compact library of mass spectra.

Keywords APCI-MS · Triacylglycerols · Triglycerides · Regioisomers · Simulacrum · Mass spectrometry

Abbreviations

A	Arachidic acid (20:0) or generic A
B	Behenic acid (22:0) or generic B
Ce	Cerotic acid (26:0)
G	Gadoleic acid (20:1)
L	Linoleic acid (18:2)
Lg	Lignoceric acid (24:0)
Ln	Linolenic acid (18:3)
M	Myristic acid (14:0)
O	Oleic acid (18:1)
P	Palmitic acid (16:0)
Po	Palmitoleic acid (16:1)
S	Stearic acid (18:0)
ACN	Acetonitrile
APCI	Atmospheric pressure chemical ionization
API	Atmospheric pressure ionization
APPI	Atmospheric pressure photoionization
BUS	Bottom Up Solution
CL	Critical Limit(s)
CR	Critical Ratio(s)
CV	Critical Value(s)
DAG	Diacylglycerol
$[DAG]^+$	Diacylglylcerol-like fragment ion, $=[M+H–RCOOH]^+$
DCM	Dichloromethane
ESI	Electrospray ionization
FA	Fatty acid(s)

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FD	First Decrement or First Deconstruction
FGFS	First General Form of a Simulacrum
FI	First Increment
FSFS	First Specified Form of a Simulacrum
FuGFS	Full General Form of a Simulacrum
GFAS	General Form of an Anti-Simulacrum
HPLC	High-performance liquid chromatography
IUS	Infinity Unit Simulacrum
MAG	Monoacylglycerol(s)
$[MAG]^+$	Monoacylglycerol-like fragments, $=[RCO + 74]^+$
MALDI-TOF	Matrix-assisted laser desorption ionization time-of-flight
MeOH	Methanol
MS	Mass spectrometry
MSS	Mass Spectrometry Simulacrum
$[MH]^+$	Protonated molecule, $=[M+H]^+$
Poss2Obs	Possibilities to observe
PtdEtn	Phosphatidylethanolamine
SFA	Saturated fatty acid(s)
SimSum	Simulacrum sum
SSFS	Second Specified Form of a Simulacrum
sn	Stereospecific numbering
TAG	Triacylglycerol(s)
UAS	Unit Anti-Simulacrum
UBUS	Updated Bottom Up Solution
UIS	Unit Identity Simulacrum
US	Unit Simulacrum
UUS	Unit Unit Simulacrum

Introduction

Triacylglycerols (TAG) are synthesized in plants and animals with fatty acids (FA) or, more precisely, fatty acyl chains, located on specific positions of the three-carbon glycerol backbone, namely *sn*-1, *sn*-2, and *sn*-3, using stereospecific numbering (*sn*). The different stereospecific molecular forms of TAG are referred to as regioisomers. In cocoa butter and vegetable oils, unsaturated FA (e.g., oleic and linoleic acids, 18:1 and 18:2, respectively) are mainly esterified at the *sn*-2 position [1–3], while saturated FA (SFA) occur preferentially at either the *sn*-1 position [3] or at the *sn*-1 and *sn*-3 positions [1, 2]. Conversely, in fats of animal origin, specifically bovine milk and pork fat (lard), SFA are esterified mainly at the *sn*-2 position, although in beef fat (tallow), SFA are esterified predominantly at the *sn*-1 and *sn*-3 positions [2]. Complementary to this, lipases in the human body show regiospecificity in the metabolism of TAG. For instance, pancreatic lipase hydrolyzes TAG primarily at the *sn*-1 and *sn*-3 positions, yielding 2-monoacylglycerols (2-MAG) and free FA [4]. Because of this, there has been interest for decades in using mass spectrometry (MS) to identify specific regioisomers.

Atmospheric pressure chemical ionization (APCI) MS has proved to be very useful for identification of regioisomers, because loss of the FA at *sn*-2 is energetically disfavored, giving smaller than expected $[AA]^+$ diacylglycerol-like fragment ions, $[DAG]^+$, for ABA TAG, versus AAB and BAA TAG [5, 6]. These are collectively referred to by Byrdwell [7, 8] as Type II TAG, since they contain two different FA. Similarly, Type III TAG (ABC/CBA/BAC/CAB/ACB/BCA) contain three different FA. The use of A, B, and C throughout this report represents generic FA, except in specific TAG names in Table 1, where A and B refer to arachidic and behenic acids, respectively. APCI-MS allows the smallest $[DAG]^+$ to be assigned as the $[sn\text{-}1,3\ AC]^+$ regioisomers [6]. Jakab et al. [9] and Fauconnot et al. [10] used these trends to great effect to construct calibration lines to quantify regioisomers. Byrdwell later showed [8, 11] that the calibration process could be simplified to use only two pure regioisomers for TAG regioisomer quantification by APCI-MS, as well as atmospheric pressure photoionization (APPI) MS and electrospray ionization (ESI) MS [12]. Byrdwell has repeatedly shown [8, 11] that tabulated values from different instruments gave similar ratios of $[DAG]^+$ abundances. Holcapek et al. [13] produced the most useful and comprehensive tabulation of raw abundances for TAG regioisomers to date, which Byrdwell converted into ratios for regioisomer quantification and identification of new trends [7].

It should be noted that non-linear calibration plots, based on $[MAG]^+$, have been reported using ESI–MS^3 of lithium adducts of some TAG [14–16] (while other TAG gave linear plots using the same technique [16]). Thus, for highly accurate quantification of regioisomers by ESI–MS^3, multipoint calibration plots made from mixtures of pure regioisomers in varying proportions should be constructed for every TAG regioisomer quantified. Unfortunately, since few pure regioisomer standards are commercially available, this requires synthesis of sets of TAG regioisomers for every TAG quantified, which is why this approach has so far been used to quantify only a small subset of regioisomers in real samples.

Another important trend identified in the first report of HPLC-APCI-MS for TAG analysis [17] was the fact that the amount of protonated molecule, $[MH]^+$, relative to the $[DAG]^+$ abundances was proportional to the degree of unsaturation of TAG, with polyunsaturated TAG giving a $[MH]^+$ base peak (=100 % relative abundance), while saturated TAG gave little or no $[MH]^+$, and a $[DAG]^+$ base peak. Byrdwell [8] constructed the $[MH]^+/\Sigma[DAG]^+$ ratio, defined as Critical Ratio 1 (CR1), to represent this trend, and showed that the average ratio approximated a sigmoidal function [7].

Table 1 Response-factor-adjusted percentage composition and isotope-adjusted Critical Ratios for soybean oil from vitamin D_3 supplement gelcap determined by APCI-MS on the TSQ Vantage EMR mass spectrometer

TAG[a]	RT	% Comp	$[MH]^+/\Sigma[DAG]^+$	$[AA]^+/[AB]^+$ or $[AC]^+/([AB]^+ + [BC]^+)$	$[BC]^+/[AB]^+$	Case
LnLnLn	46.99	0.04	6.2769			2
LnLLn	50.05	0.80	4.1648	0.5098		2.1
LLnL	53.68	5.75	3.3643	0.4793		2.1
LnOLn	54.23	0.53	7.0533	0.5257		2.1
LnLM	55.86	0.04	1.8822	0.1542	0.2296	2.1.1
LnLnP	56.32	0.16	3.4844	1.3111		2.2
LLL	57.97	17.30	2.3099			2
OLnL	58.97	3.79	1.9278	0.3035	0.9079	2.1.1
LnOPo	59.56	0.03	0.2419	0.0374	0.0407	1.1.1
LLM	60.74	0.24	1.1666	2.3016		2.2
LnLP	61.17	2.93	1.9678	0.2523	0.4943	2.1.1
PLnPo	61.83	0.01	0.2569	0.0411	0.1339	1.1.1
LnOM	61.91	0.02	0.8368	0.0743	0.1613	2.1.1
LLO	64.16	15.83	1.1816	0.8269		2.1
OLnO	65.33	0.96	1.3735	0.2988		2.1
OLPo	65.40	0.09	0.3180	0.0960	0.3497	1.1.1
LLP	66.76	13.88	1.1224	1.2961		2.2
PoLP	67.90	0.10	0.0848	0.0602	0.5568	1.1.1
OLnP	68.01	0.89	1.0254	0.2651	0.4016	2.1.1
LLG	69.67	0.23	0.9881	1.9864		2.2
PLM	70.66	0.04	0.0276	0.2596	0.7985	1.1.1
PLnP	70.93	0.12	0.4795	0.2604		1.1
OLO	71.44	6.90	0.4652	0.2931		1.1
LLS	73.37	4.22	1.0579	1.1257		2.2
LLnA[b]	73.56	0.09	1.1998	0.2780	0.3980	2.1.1
OOPo	73.79	0.09	0.3973	1.6860		1.2
SLnO	74.53	2.29	0.0774	0.0205	0.0515	1.1.1
OLP[b]	74.54	8.05	0.2581	0.3024	0.4677	1.1.1
PoOP	75.88	0.02	0.0387	0.1704	0.7904	1.1.1
OLG	77.77	0.16	0.2640	0.1026	0.3354	1.1.1
PLP	77.94	2.01	0.0280	0.3122		1.1
OOO	79.86	1.80	0.1485			1
LLnB	80.43	0.10	1.6379	0.3037	0.7259	2.1.1
LLA	80.47	0.33	1.0735	1.1827		2.2
LGP	81.25	0.11	0.8049	0.3056	0.8005	2.1.1
OLS	82.00	2.87	0.2528	0.3039	0.5057	1.1.1
OOP	83.56	1.89	0.0879	0.6910		1.1
LL-21	84.12	0.07	0.9640	1.6462		2.2
SLP	85.88	1.37	0.0278	0.3371	0.8298	1.1.1
POP	87.65	0.47	0.0403	0.2057		1.1
LLB	87.91	0.38	1.0399	1.0185		2.2
OLA	89.84	0.17	0.3150	0.2833	0.5430	1.1.1
PGO	91.01	0.03	0.1645	0.1877	0.2408	1.1.1
OOS	91.69	0.73	0.0782	0.5506		1.1
PPP	93.83	0.00	0.0026			1
LO-21	93.85	0.02	0.2487	0.2899	0.4070	1.1.1
PAL	94.19	0.24	0.0615	0.2302	0.3902	1.1.1

Table 1 continued

TAG[a]	RT	% Comp	$[MH]^+/\Sigma[DAG]^+$	$[AA]^+/[AB]^+$ or $[AC]^+/([AB]^+ + [BC]^+)$	$[BC]^+/[AB]^+$	Case
SLS	94.25	0.29	0.0348	0.5003		1.1
LLLg	95.64	0.12	1.0648	0.9541		2.1
SOP	96.28	0.39	0.0412	0.1587	0.8197	1.1.1
OLB	97.84	0.18	0.2981	0.2995	0.5988	1.1.1
GSO	99.24	0.01	0.1229	0.3856	0.8542	1.1.1
LL-25	99.59	0.00	0.9761	1.4217		2.2
OOA	100.10	0.05	0.1021	0.5691		1.1
OL-23[b]	101.89	0.01	0.2549	0.3140	0.4771	1.1.1
LBP	102.28	0.16	0.0357	0.3742	0.9566	1.1.1
ASL	102.56	0.08	0.0542	0.2899	0.4318	1.1.1
PPS	103.22	0.00	0.0014	0.6054		1.1
LLCe	103.46	0.00	1.0217	1.1558		2.2
OO-21	104.29	0.01	0.0692	0.9082		1.1
PAO	104.99	0.07	0.1139	0.3514	0.7378	1.1.1
SOS	105.09	0.10	0.0507	0.2388		1.1
OLLg[b]	105.79	0.06	0.2777	0.3314	0.6022	1.1.1
OOB	108.39	0.05	0.0852	0.5080		1.1
PLgL	110.76	0.05	0.0338	0.3500	0.8779	1.1.1
LBS	111.33	0.05	0.0297	0.4422	0.9741	1.1.1
BOP	113.04	0.04	0.0503	0.3664	0.7298	1.1.1
OAS	113.32	0.02	0.0675	0.4878	0.9722	1.1.1
OOLg	114.75	0.02	0.0720	0.5187		1.1
LgLS	115.88	0.02	0.0250	0.4647	0.8958	1.1.1
OLgP	116.72	0.02	0.0466	0.4291	0.8727	1.1.1
BOS	116.93	0.01	0.0419	0.3430	0.8758	1.1.1
OOCe	117.52	0.00	0.0495	0.7782		1.1
LgOS	118.87	0.01	0.0283	0.2938	0.9231	1.1.1
Sum		100.00	Avg.: 0.7746			

The following FA abbreviations are used (carbon chain length:sites of unsaturation): *M,* myristic acid (14:0); *Po,* palmitoleic acid (16:1); *P,* palmitic acid (16:0); *Ln,* linolenic acid (18:3); *L,* linoleic acid (18:2); *O,* oleic acid (18:1); *S,* stearic acid (18:0); *G,* gadoleic acid (20:1); *A,* arachidic acid (20:0); *B,* behenic acid (22:0); *Lg,* lignoceric acid (24:0); *Ce,* cerotic acid (26:0)

[a] TAG name indicates most abundant regioisomer, as indicated by Critical Ratio 2, and order of fatty acyl chains indicated by Critical Ratio 3 set to less than 1

[b] Order of FA changed after isotope correction, discussed thoroughly by Holcapek et al. [13]. Bold indicates clean spectrum. Italics indicate spectrum contains substantial peaks from more than one TAG. Underline indicates different assignment from UBUS report

Based on the trends above, Byrdwell constructed three "Critical Ratios" to describe different aspects of TAG structural analysis [8]. As mentioned, the $[MH]^+/\Sigma[DAG]^+$ ratio was defined as Critical Ratio 1, and was used to describe the correlation of $[MH]^+$ and $[DAG]^+$ abundances to the degree of unsaturation. The second ratio that was defined, Critical Ratio 2, was the $[AA]^+/[AB]^+$ ratio for Type II TAG, and the analogous $[AC]^+/([AB]^+ + [BC]^+)$ ratio for Type III TAG, which were used for regioisomer identification and quantification. The third ratio, Critical Ratio 3, was the $[BC]^+/[AB]^+$ ratio, and applied only to Type III TAG. For two decades it was reported that no trends were observable for the fragmentation behavior of $[AB]^+$ versus $[BC]^+$, and that APCI-MS could not differentiate these fragments. Byrdwell hypothesized [8]: "if one chooses to set the $[BC]^+$ fragment equal to the smaller of the two possible abundances for $[AB]^+$ or $[BC]^+$, then perhaps the ratios will indicate some structural trends". Using this convention to assign $[BC]^+/[AB]^+$, Byrdwell [7] identified trends in 25 of 27 TAG in the data tabulated by Holcapek et al. [13], and showed that the predominant factor in fragment abundances was the degree of unsaturation and grouping of FA on the glycerol backbone. Fragment abundances were larger for $[DAG]^+$ that had two polyunsaturated FA adjacent, whereas when they were separated by a saturated

or monounsaturated FA, loss of the polyunsaturated FA became the predominant mechanism.

The Bottom Up Solution (BUS) [8] and the Updated Bottom Up Solution (UBUS) [7] showed how Critical Ratios could be used for elucidation of structural information from APCI-MS of TAG. They also showed that the Critical Ratios constituted a "reduced data set" from which the raw mass spectral abundances could be reconstructed at will by processing the Critical Ratios through the BUS or the UBUS to reproduce the mass spectra. This means that the Critical Ratios provide the structural information desired, and also constitute a library of mass spectra. Based on the diagram of the BUS and UBUS that appeared as half of an octahedron, it became apparent that the missing half of the octahedron represented ratios that were ignored, since they did not most directly provide the structural information desired. For instance, the $[AA]^+/[AB]^+$ ratio was used, but the $[AB]^+/[AA]^+$ ratio could have been used; it simply was not as direct and convenient for construction of the calibration lines. If the BUS and UBUS are generalized to include all possible options, the resultant all-inclusive construct is what is defined here as a simulacrum. The name simulacrum comes from the philosophical text by Jean Baudrillard, Simulacrum and Simulation [18]. The meaning of "simulacrum" as used here is a construct that is so thorough and complete in representing a system it describes as to render the original system unnecessary (superseding the original), as in the case of needing fewer Critical Ratios to represent the abundances in a mass spectrum, while at the same time providing more information in those fewer values.

Herein is described the form and function of a new mathematical construct known as a simulacrum and its first application, to APCI-MS. It is demonstrated to be the construct behind the previously published BUS and UBUS. It is further shown to be a construct that is so generalized that it can be applied to mass spectrometry of other lipids, as well as non-lipids, and is possibly a universal function applicable to any values or ratios from $-\infty$ to ∞.

Materials and Methods

Solvents and Samples

Methanol (MeOH), acetonitrile (ACN), and dichloromethane (DCM) were Optima LC–MS grade (Thermo Fisher Scientific, Inc., Fairlawn, NJ, USA). Ammonium formate was from Sigma Aldrich, Inc. (St. Louis, MO, USA), and was supplied as the electrolyte for ESI–MS, in the form of a 50 mM solution in H_2O/ACN (1:4) at 20 μL/min via an ABI 140B (Applied Biosystems, Inc., Foster City, CA, USA) dual piston syringe pump. Samples were dietary supplements containing vitamin D_3 dissolved in a vegetable oil, which were purchased from an online supplier of herbal supplements, kept refrigerated, and used before their expiration date.

Liquid Chromatography

High performance liquid chromatography (HPLC) was conducted using an Agilent 1200 system (Agilent Technologies, Santa Clara, CA, USA) with UV detector, corona charged aerosol detector (CAD; Thermo Scientific Dionex, Sunnyvale, CA, USA), and evaporative light scattering detector (ELSD; Agilent Technologies, Santa Clara, CA, USA), as previously described [19]. Non-aqueous reversed-phase (NARP) HPLC was conducted using two Inertsil ODS-2 C18 columns (25.0 cm × 4.6 mm, 5 μm, GL Sciences, Torrance, CA, USA) in series maintained at 10 °C. 20 μL of ~1 mg/mL solutions were injected. The separation of vitamin D was done using 100 % methanol followed by an ACN/DCM gradient.

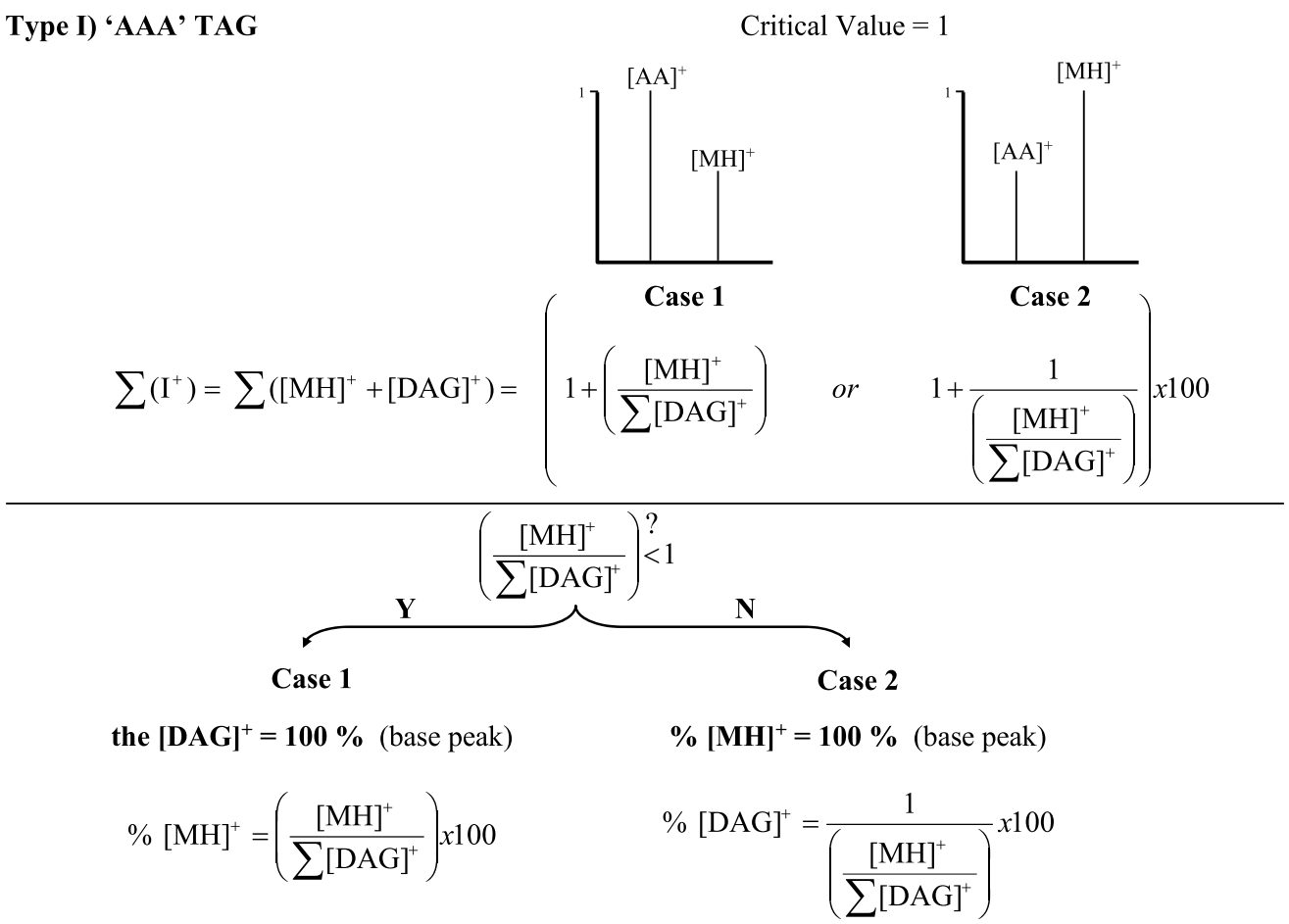
Mass Spectrometry

Three mass spectrometers, in parallel, were used for this analysis. A ThermoScientific (San Jose, CA, USA) TSQ Vantage EMR tandem sector quadrupole (TSQ) mass spectrometer was used for APCI-MS with the conditions previously described [20], a ThermoScientific LCQ Deca XP ion trap mass spectrometer was used for ESI–MS with the conditions previously described [19], and an older ThermoScientific TSQ 7000 mass spectrometer was used as a secondary APCI-MS detector with the conditions previously described [20].

Note that the proper names of specifically defined entities, such as Critical Ratios and Cases, are capitalized. In the examples below, a Type I TAG is mono-acid TAG, AAA, which produces a diacylglycerol-like fragment $[AA]^+$. This is not to be confused with generic ions $[A]^+$ and $[B]^+$.

Results

Most of the principles involved with the simulacrum system can be demonstrated using the simple mass spectra of Type I TAG. Scheme 1 from the UBUS is repeated as Scheme 1 here. The mass spectrum of a Type I TAG shows only the single diacylglycerol-like fragment ion, $[DAG]^+$, and the protonated molecule, $[MH]^+$ (plus isotope peaks). As shown before, the ratio of these ions, known as Critical Ratio 1, depends on the degree of unsaturation, with polyunsaturated TAG having an $[MH]^+$ base peak, while saturated TAG give little or no $[MH]^+$. Since the $[MH]^+$ can be zero for saturated TAG, Byrdwell chose to construct the $[MH]^+/\Sigma[DAG]^+$ ratio instead of



Scheme 1 Equations to calculate the relative abundances of the $[MH]^+$ ion and the $[DAG]^+$ fragment ion for a Type I TAG using the $[MH]^+/\Sigma[DAG]^+$ Critical Ratio in Table 1

the $\Sigma[DAG]^+/[MH]^+$ ratio to avoid the possibility of a "divide by zero" error [8]. However, the possibility exists to use the $\Sigma[DAG]^+/[MH]^+$ ratio instead, in cases where the $\Sigma[DAG]^+$ might be zero, such as MALDI-TOF–MS of TAG [21]. Also, it is appreciated that other instruments may employ different collision-induced dissociation (CID) energies that lead to different ratios. Therefore, it is desirable to generalize the equations used, to allow for all possibilities. Before presenting the generalized equation, an important component of the BUS and the UBUS will be mentioned, which was testing the Critical Ratios against Critical Values (CV) and Critical Limits (CL) to determine which ion was the base peak. In mass spectrometry, the base peak is assigned a value of 100 percent. Since "percent" means "per hundred", 100 percent has a value as a pure fraction of 100 per 100, or 1. As seen below, identifying which ion has a value of 1 is the key to simplifying the equations by cancelling out the 1, since 1 times any value or ratio is equal to the value or ratio (i.e., the identity property of multiplication). Before providing mathematical examples, it is necessary to define three simulacrum constructs.

The First General Form of a Simulacrum (FGFS)

A simulacrum sum is equal to the mathematical sum of two values, but that sum is expressed as a product of a value and a ratio. In the case of the BUS and UBUS, the value is the abundance of the base peak (100 % = 1), determined by classifying the ratios, and the ratio is one of the Critical Ratios that provides the desired structural information, described previously. The components of the First General Form of a Simulacrum (FGFS) of two values A and B are shown in Fig. 1, and are as follows: (1) the simulacrum sum, or SimSum(A,B), which represents all possible ways A and B can be expressed as a value and a ratio; (2) the mathematical sum, which, for mass spectrometry, is equal to the sum of ions; (3) the "Possibilities to Observe" (PossObs or Poss2Obs), which shows the two possible values and the two possible ratios that can be constructed from

Fig. 1 The First General Form of a Simulacrum (FGFS), where A and B can represent any values, and the default Case 1 has A ≤ B. A simulacrum is composed of the Simulacrum Sum, Possibilities to Observe, two Cases, and eight solutions

$$\text{Simulacrum Sum (A,B)} = \text{SimSum(A,B)} = \sum_{\text{2-0-A-B}}(\text{A}+\text{B}) =$$

Possibilities to Observe:

$$\begin{matrix}\text{A} & \text{B}\\ \left(\frac{\text{A}}{\text{B}}\right) & \left(\frac{\text{B}}{\text{A}}\right)\end{matrix}$$

$$\left[\begin{array}{l}
\text{Case 1}: \text{A} \le \text{B}, \text{B} \ge \text{A}, \left(\frac{\text{A}}{\text{B}}\right) \le 1, \left(\frac{\text{B}}{\text{A}}\right) \ge 1; \\
\text{A}\left(1+\frac{1}{\left(\frac{\text{A}}{\text{B}}\right)}\right) \quad \text{or} \quad \text{B}\left(1+\left(\frac{\text{A}}{\text{B}}\right)\right) \\
\text{A}\left(1+\left(\frac{\text{B}}{\text{A}}\right)\right) \quad \text{or} \quad \text{B}\left(1+\frac{1}{\left(\frac{\text{B}}{\text{A}}\right)}\right) \\
\text{Case 2}: \text{A} \ge \text{B}, \text{B} \le \text{A}, \left(\frac{\text{A}}{\text{B}}\right) \ge 1, \left(\frac{\text{B}}{\text{A}}\right) \le 1; \\
\text{A}\left(1+\frac{1}{\left(\frac{\text{A}}{\text{B}}\right)}\right) \quad \text{or} \quad \text{B}\left(1+\left(\frac{\text{A}}{\text{B}}\right)\right) \\
\text{A}\left(1+\left(\frac{\text{B}}{\text{A}}\right)\right) \quad \text{or} \quad \text{B}\left(1+\frac{1}{\left(\frac{\text{B}}{\text{A}}\right)}\right)
\end{array}\right]$$

those two values; (4) the two Cases, which classify the values and ratios as A less than or equal to B, so (A/B) ≤ 1, or A greater than or equal to B, so (A/B) ≥ 1; and (5) the eight solutions, four for Case 1 and four for Case 2, which represent all the ways that the solution can be obtained using one value and one ratio. Note that the four Case 1 solutions are identical in form to the four Case 2 solutions.

Since any simulacrum contains two Cases, a lower Case and a higher (upper) Case, and contains eight solutions, it can be envisioned as an octahedron, Fig. 2. The four lower sides are the four Case 1 solutions, the four upper sides are the four Case 2 solutions, and the four equatorial lines that are shared by the lower and upper sides are the four equalities arising from using "less than or equal to" and "greater than or equal to".

As shown in Fig. 1, for values A and B, the SimSum(A,B) equals Sum(A + B), which has the possibility to observe directly the value of A, the value of B, or to observe the ratio of (A/B), or the ratio of (B/A). Then, Case 1 is A less than or equal to B, so (A/B) ≤ 1, and Case 2 is A greater than or equal to B, so (A/B) ≥ 1. Once classified into a Case, the solution can be found using whichever of the four solutions is most convenient, or whichever one there is enough information provided to

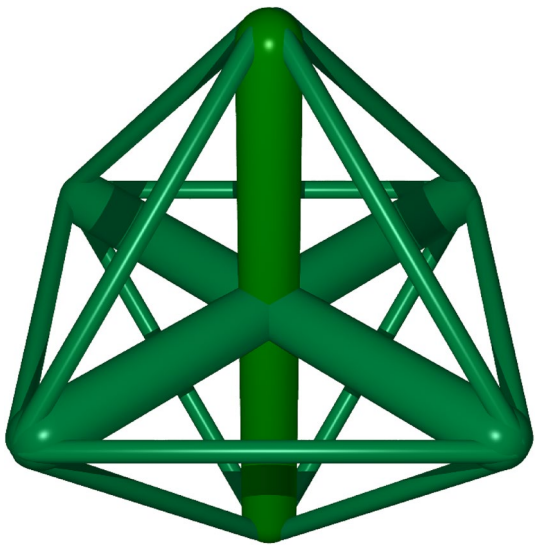


Fig. 2 The shape of a simulacrum. A simulacrum is composed of two Cases, a lower Case (1) and a higher Case (2), represented by the upper and lower halves of the octahedron. Each Case has four solutions, represented by the four lower sides of the octahedron (Case 1) and the four upper sides of the octahedron (Case 2). The four equatorial lines represent the equalities in the Case classification, in which either Case 1 or Case 2 provides equal solutions if A = B, and (A/B) = 1

$$\text{Simulacrum Sum } ([MH]^+, [DAG]^+) = \text{SimSum}([MH]^+, [DAG]^+) = \sum_{\text{2-0-}[MH]^+\text{-}\sum[DAG]^+} \left([MH]^+ + \sum [DAG]^+ \right) =$$

Possibilities to Observe:

$[MH]^+$ $[DAG]^+$

$$\left(\frac{[MH]^+}{\sum [DAG]^+} \right) \quad \left(\frac{\sum [DAG]^+}{[MH]^+} \right)$$

$$\begin{cases} \text{Case 1}: [MH]^+ \le \sum [DAG]^+, \sum [DAG]^+ \ge [MH]^+, \\ \left(\frac{[MH]^+}{\sum [DAG]^+} \right) \le 1, \left(\frac{\sum [DAG]^+}{[MH]^+} \right) \ge 1; \\ [MH]^+ \left(1 + \frac{1}{\left(\frac{[MH]^+}{\sum [DAG]^+} \right)} \right) \quad \text{or} \quad \sum [DAG]^+ \left(1 + \left(\frac{[MH]^+}{\sum [DAG]^+} \right) \right) \\ [MH]^+ \left(1 + \left(\frac{\sum [DAG]^+}{[MH]^+} \right) \right) \quad \text{or} \quad \sum [DAG]^+ \left(1 + \frac{1}{\left(\frac{\sum [DAG]^+}{[MH]^+} \right)} \right) \\ \text{Case 2}: [MH]^+ \ge \sum [DAG]^+, \sum [DAG]^+ \le [MH]^+, \\ \left(\frac{[MH]^+}{\sum [DAG]^+} \right) \ge 1, \left(\frac{\sum [DAG]^+}{[MH]^+} \right) \le 1; \\ [MH]^+ \left(1 + \frac{1}{\left(\frac{[MH]^+}{\sum [DAG]^+} \right)} \right) \quad \text{or} \quad \sum [DAG]^+ \left(1 + \left(\frac{[MH]^+}{\sum [DAG]^+} \right) \right) \\ [MH]^+ \left(1 + \left(\frac{\sum [DAG]^+}{[MH]^+} \right) \right) \quad \text{or} \quad \sum [DAG]^+ \left(1 + \frac{1}{\left(\frac{\sum [DAG]^+}{[MH]^+} \right)} \right) \end{cases}$$

Fig. 3 The First General Form of a Simulacrum (FGFS), where $[MH]^+$ and $\Sigma[DAG]^+$ can represent any values, and the default Case 1 has $[MH]^+ \le \Sigma[DAG]^+$. A simulacrum is composed of the Simulacrum Sum, Possibilities to Observe, two Cases, and eight solutions

solve. The FGFS with A and B replaced with $[MH]^+$ and $\Sigma[DAG]^+$ is given in Fig. 3. When applied to the example of Scheme 1, $\Sigma[DAG]^+$ is simply $[AA]^+$, and the SimSum($[MH]^+$,$\Sigma[DAG]^+$) equals the sum of all ions, $\Sigma(I^+)$.

Additional nomenclature for the simulacrum system is as follows: the "2-0-A-B" below the sum (Σ) symbol (Fig. 1) indicates "dimensions − time − variable 1 − variable 2", where two values are being used, indicating a two-dimensional variable space, time is not being monitored or used in this first application, and variable 1 and variable 2 are A and B, respectively. Time could be a chromatographic retention time or other time variable, which we will ignore (set = 0) in this first report, although it is identified and kept open for use in future applications. Unless otherwise specified, A and B can be reversed, so $\Sigma(A + B) = \Sigma(B + A)$, using the commutative property of addition. Also, in the special case of mass spectrometry, the dimensionality equals the maximum value of the sum, if all abundances are at their maximum values of 1 (100 %), which occurs at the Critical Values previously described [7, 8].

Several important points can be made about the FGFS in Fig. 1. First, there are no limitations placed on the values of A and B. They could be any number from $-\infty$ to

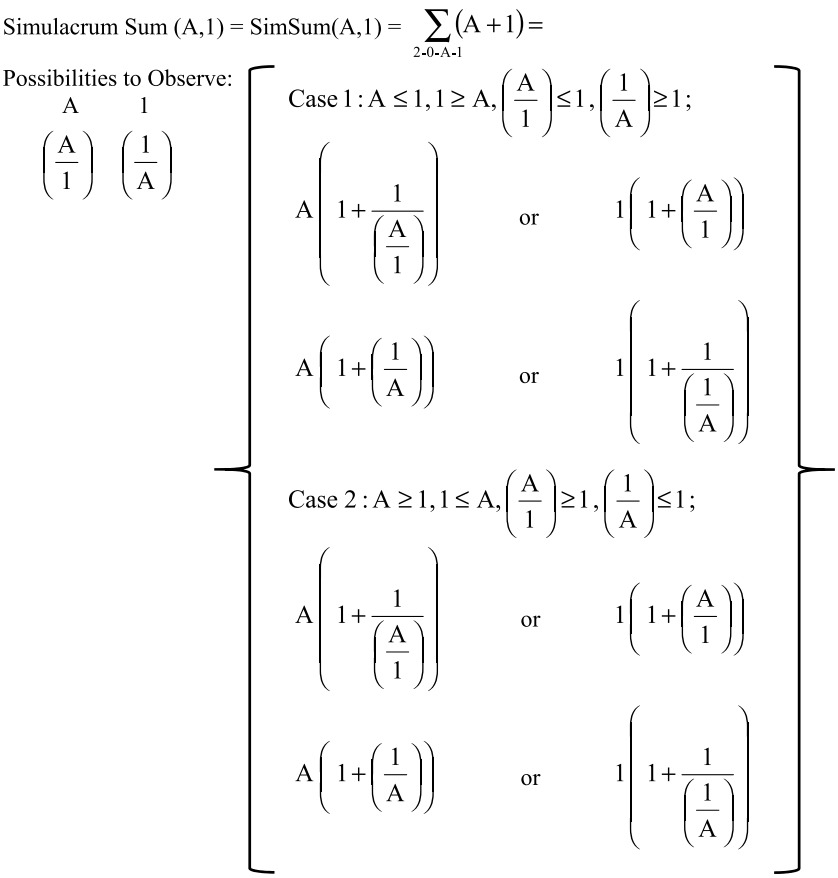


Fig. 4 The Unit Simulacrum (US), or First Specified Form of a Simulacrum (FSFS) where one value is specified to be 1, and the other value, A, can represent any value. The default Case 1 has A ≤ 1. A simulacrum is composed of the Simulacrum Sum, Possibilities to Observe, two Cases, and eight solutions

$+\infty$. Furthermore, A and B don't really even need to be numbers. Because of the way things cancel out in the eight solutions in the two Cases, A and B can actually be any number or symbol. For instance, A could be ♣ and B could be ♥, and by doing the appropriate cancelling out in the eight solutions, the SimSum(♣,♥) equals Sum(♣ + ♥), determined from the Poss2Obs: ♣, ♥ (♣/♥), (♥/♣). Thus, the FGFS appears to apply to anything that has a number, symbol, name, or can be written in physical form, except two zeros. The meaning associated with a ratio such as (♣/♥) does not depend on the simulacrum itself, which is just a framework. The meaning assigned to the symbols or ratios of symbols is whatever interpretation we choose to associate with that (those) symbol(s). The set of factors that provide the association of meaning with whatever symbol or ratio is used in a simulacrum is called the Interpretation Matrix [7, 8]. In the case of the BUS and the UBUS, the Interpretation Matrix was made of the associations of Critical Ratios with the structural information they provide based on literature precedent (i.e., Critical Ratio 1 is related to the degree of TAG unsaturation, Critical Ratio 2 is related to identification and quantification of regioisomers, and Critical Ratio 3 is related to the degree of unsaturation and grouping of FA), as previously discussed [7, 8].

The Unit Simulacrum (US)

No limitations were placed on the FGFS. The first limitation we want to impose on the FGFS is to specify that one value is 1, to align with the principle of mass spectrometry that one value is 1, the base peak. This first specification gives the First Specified Form of a Simulacrum (FSFS), Fig. 4, also referred to as the Unit Simulacrum (US), since it contains the first specified unit, or 1. This becomes the first part of the Interpretation Matrix for the US: one value is 1. If we start with the FGFS in Fig. 1 and replace every "B" with "1", we obtain SimSum(A,1) = Sum(A + 1) = Sum(1 + A), the Unit Simulacrum for A. Alternatively, we could replace every "A" with "1" to obtain SimSum(1,B) = Sum(1 + B) = Sum(B + 1), which is given in Online Resource 1.

One key aspect of the BUS and the UBUS was to use the classification process (based on Critical Values and Critical Limits) to determine which ion was the "unit" (=1), so when the 1 was multiplied times what was inside the set of parentheses, the solution simply became whatever was inside the parentheses (based on the identity property of multiplication), giving a simple 1 + ratio or 1 + 1/ratio, which half of the solutions devolved to. Thus, half of the solutions devolved to a US.

$$\text{Simulacrum Sum } ([A]^+,[B]^+) = \text{SimSum}([A]^+,[B]^+) = \sum_{2\text{-}0\text{-}[A]^+\text{-}[B]^+} \left([A]^+ + [B]^+\right) =$$

Possibilities to Observe: $[A]^+$ $[B]^+$ $\left(\frac{[A]^+}{[B]^+}\right)$ $\left(\frac{[B]^+}{[A]^+}\right)$

$$\left\{\begin{array}{l}
\text{Case 1}: [A]^+ \le [B]^+, [B]^+ = 1, \left(\frac{[A]^+}{[B]^+}\right) \le 1, \left(\frac{[B]^+}{[A]^+}\right) \ge 1; \\
[A]^+\left(1+\frac{1}{\left(\frac{[A]^+}{[B]^+}\right)}\right) \quad \text{or} \quad 1\left(1+\left(\frac{[A]^+}{[B]^+}\right)\right) \\
[A]^+\left(1+\left(\frac{[B]^+}{[A]^+}\right)\right) \quad \text{or} \quad 1\left(1+\frac{1}{\left(\frac{[B]^+}{[A]^+}\right)}\right) \\
\text{Case 2}: [A]^+ = 1, [B]^+ \le [A]^+, \left(\frac{[A]^+}{[B]^+}\right) \ge 1, \left(\frac{[B]^+}{[A]^+}\right) \le 1; \\
1\left(1+\frac{1}{\left(\frac{[A]^+}{[B]^+}\right)}\right) \quad \text{or} \quad [B]^+\left(1+\left(\frac{[A]^+}{[B]^+}\right)\right) \\
1\left(1+\left(\frac{[B]^+}{[A]^+}\right)\right) \quad \text{or} \quad [B]^+\left(1+\frac{1}{\left(\frac{[B]^+}{[A]^+}\right)}\right)
\end{array}\right\}$$

Fig. 5 The Second Specified Form of a Simulacrum (SSFS), or Mass Spectrometry Simulacrum (MSS), where either A or B can be 1, and no value can be greater than 1. The default Case 1 has $[A]^+ \le 1$. A Unit Simulacrum is composed of the Simulacrum Sum, Possibilities to Observe, two Cases, and eight solutions. In the MSS, the larger ion is set to 1 (=100 %), the base peak

It is interesting to note that every solution for both the FGFS and the US (and, in fact, every simulacrum) included a US, i.e. 1 + ratio or 1 + 1/ratio, inside the parentheses. Thus, there is a self-similarity inherent in the simulacrum system. The "1" inside the parentheses is different from the "1" outside the parentheses arising from mass spectrometry, or from setting one value to one. Thus, there is always a "1" present in every simulacrum, and so there is always a US present (inside the parentheses), regardless of the values of the variables.

It is also worth mentioning that, just like the FGFS, the US can apply to any defined entity, including names, numbers, symbols, or anything that can be written. The solutions in Fig. 4 hold true for anything that can be written in place of "A", except zero.

The Mass Spectrometry Simulacrum (MSS)

In the US, while one value was specified to be "1", the other value could be anything. In mass spectrometry, no value (abundance) can be greater than 1 (100 %). Thus, mass spectrometry imposes a second specification on the simulacrum system, which is that no value can be greater than 1. Therefore, a simulacrum constrained by these two specifications results in the Second Specified Form of a Simulacrum (SSFS), also called the Mass Spectrometry Simulacrum (MSS), which is shown in Fig. 5. The Interpretation Matrix for the MSS, therefore, has two initial components: one value is 1, and no value can be greater than 1. Because of this second specification, the SSFS or MSS in Fig. 5 now includes $[B]^+ = 1$ in the Case 1 definition and $[A]^+ = 1$ in the Case 2 definition.

With the FGFS, FSFS, and SSFS defined, we are now in a position to arrive at all of the solutions presented previously in the BUS and the UBUS. If we let A = $[MH]^+$ and B = $[AA]^+ = \Sigma[DAG]^+$ for a Type I TAG, we can demonstrate what happens when each of these is set equal to the base peak, 100 % = 1. For instance, in Table 1 trilinolein, LLL, has an $[MH]^+/\Sigma[DAG]^+ = 2.3099$, which Fig. 3 (and Scheme 1) shows is Case 2, so the $[MH]^+$ fragment = 1, and the solution shown in Fig. 3 is $[MH]^+(1 + 1/([MH]^+/\Sigma[DAG]^+))$, which becomes $1(1 + 1/([MH]^+/\Sigma[DAG]^+))$. We ignore the "1" multiplying outside the parentheses to obtain the Case 2 sum given in Scheme 1, and if $[MH]^+ = 1$, then $\Sigma[DAG]^+ = [LL]^+ = 1/([MH]^+/\Sigma[DAG]^+) = 1/2.3099 = 0.4329$, or 43.29 %. Similarly, triolein, OOO, has a CR1 of 0.1485, which Fig. 3 (and Scheme 1) shows is Case 1, so the solution in Fig. 3 is $\Sigma[DAG]^+(1 + [MH]^+/\Sigma[DAG]^+)$, which becomes $1(1 + [MH]^+/\Sigma[DAG]^+)$. We again ignore the

"1" multiplying outside the parentheses to obtain the Case 1 sum given in Scheme 1, and if $\Sigma[DAG]^+ = [LL]^+ = 1$, then $[MH]^+ = [MH]^+/\Sigma[DAG]^+ = 0.1485$, or 14.85 %. These two examples demonstrate how the FGFS for $[MH]^+$ and the $\Sigma[DAG]^+$, Fig. 3, was really behind the solutions given in Scheme 1 in the BUS and UBUS. The simplified solutions in the BUS and UBUS were only obtained after classifying the ratio, setting one value to 1, and then ignoring that "1" to obtain the solution inside the parentheses. This shows that the BUS and the UBUS actually provided only one of the four possible Case 1 solutions, and one of the four possible Case 2 solutions, and these were the solutions that used the ratio that we chose to construct, and which were classified such that one value was 1 and could be ignored. Each term in the simulacrum sum provides the abundance of one of the ions. This is the idea behind the term-wise simulacrum solution mentioned at the end of the BUS [8].

The above example for a Type I TAG demonstrates some important principles that produce the BUS and the UBUS from the FGFS, US, and MSS. First, the primary function of the Case classification system is to narrow down the solutions to those that have a multiplying 1 outside the parentheses that can be multiplied through so it disappears, thereby simplifying the solutions. Second, the FGFS shows all possible solutions, which allows us to choose the one that is easiest to solve using whichever ratio we want to construct to provide the structural information desired. We can ignore the solutions that still require a value (A or B) *and* a ratio, and use the simplified solution that provides the answer using only the ratio.

Now that the complete framework behind the BUS and UBUS is shown, an important point about these constructs can be made. In the BUS and UBUS, a Critical Ratio was classified as Case 1 if it was less than the Critical Limit, instead of less than *or equal to* the CL, merely to minimize confusion in the implementation of the simulacrum system. Case 2 was defined as the ratio being greater than *or equal to* one so that the moment a ratio equaled one, it became Case 2. This became an inherent part of the Interpretation Matrix for the BUS and UBUS. However, all the simulacra defined here show that when A = B, exactly mathematically equal solutions are provided by both Case 1 and Case 2, and Case 1 = Case 2. Nevertheless, to avoid the uncertainty of whether $[MH]^+ = \Sigma[DAG]^+$ would be called Case 1 or Case 2, the decision was made to eliminate ambiguity in the BUS and UBUS. However, now that the fully generalized forms behind the BUS and UBUS are being defined, every possibility is allowed.

The Unit Unit Simulacrum (UUS)

If the $[MH]^+$ and $\Sigma[DAG]^+$ in Fig. 3 are both equal to 1, such as at the Critical Value, which equals the Critical Limit for a Type I TAG, then all eight solutions of Fig. 3 are equal, and the simulacrum sum equals the maximum sum of abundances, 1 + 1 = 2, shown in the BUS and UBUS (Fig. 2). Just as a simulacrum with one value of 1 was a Unit Simulacrum, the special case where both values are 1 has a special name, the Unit Unit Simulacrum (UUS), since it contains two units. Although its solution is trivial, the UUS is shown explicitly in Online Resource 2 because it holds a unique position in the overall system of simulacrum mathematics.

The Unit Identity Simulacrum (UIS)

Another special case for the application of the simulacrum system to mass spectrometry is found for saturated TAG, which can produce an $[MH]^+$ abundance of zero. This is why the $[MH]^+/\Sigma[DAG]^+$ was selected for CR1, not the $\Sigma[DAG]^+/[MH]^+$, which could cause a "divide by zero" error in calculations. In the special case of Type I saturated TAG, such as PPP and SSS, the $[DAG]^+$ is the base peak, =1, and the $[MH]^+$ is often 0. The simulacrum sum of 1 and 0 constitutes a unique entity in the simulacrum system, known as the Unit Identity Simulacrum (UIS), Online Resource 3, analogous to the identity property of addition (also called the additive identity property), in which 0 plus any number equals that number. The ratio (1/0) appears in the simulacrum. In calculators and spreadsheets, this gives a divide by zero error because, in mathematics, 1/0 is undefined. Therefore, the UIS is unique because it gives only one rational solution in each Case: $1(1 + (0/1)) = 1 + 0$. Nevertheless, the UIS shows all possible solutions, whether defined or undefined, rational or irrational.

The Anti-Simulacrum

In the discussions of all simulacra above, Case 1 was defined such that a ratio ≤ 1 is called Case 1, and Case 2 was defined such that a ratio ≥ 1 is Case 2. By doing this, the magnitude of the ratio is aligned with the magnitude of the Case number (smaller ratio, smaller Case; larger ratio, larger Case). Thus, the FGFS can also be called the Aligned General Form of a Simulacrum. But that is not the only conceivable way the construct could have been organized.

When the BUS was first discovered, I thought that the "natural" case, or most self-evident case, was where polyunsaturated TAG gave an $[MH]^+$ as the base peak, versus saturated TAG, which gave an $[MH]^+$ equal to or close to zero. When that manuscript was first submitted, it had what I thought to be the "natural" case set as Case 1, so $[MH]^+/\Sigma[DAG]^+ \geq 1$ was Case 1. However, when I saw the "bigger picture" and discovered the FGFS and the US, which simplified to give the BUS, I realized that the Case number should be aligned with the magnitude of the ratio, so the

ratio ≤1 should be Case 1 and the ratio ≥1 should be Case 2. Therefore, the magnitude of the ratio was aligned with the Case number in the revised and published report of the BUS.

However, for an all-inclusive system that encompasses *every* possibility, there exists the option to construct the inverse of the FGFS and US, in which Case 1 is defined as the ratio ≥1 and Case 2 is defined as the ratio ≤1. Defining the Cases in this way gives rise to the General Form of the Anti-Simulacrum (GFAS), shown in Online Resource 4. The GFAS can be seen to be the inverse of the FGFS in Fig. 1. Together, these two constructs represent every conceivable possibility for representing the sum of two values as the product of a value and a US of a ratio or the inverse of a ratio, whether the Cases are properly understood or misunderstood, aligned or unaligned. The sum of all possibilities represented by the combination of the FGFS and the GFAS is the most fully generalized form of a simulacrum, referred to as the Full General Form of a Simulacrum (FuGFS).

In most cases, the FuGFS will not be used. The FGFS, Fig. 1, is sufficient to use for the vast majority of applications, such as mass spectrometry, which is why the abbreviation "FGFS" was given to the First General Form of a Simulacrum, and the more awkward and unconventional abbreviation FuGFS was given to the Full General Form of a Simulacrum. Nevertheless, to account for every possibility, the GFAS and the FuGFS are defined.

An example to help understand the GFAS is as follows: Comparing Fig. 4 to Online Resource 1, "A" and "B" were defined above as being one or the other of the abundances for a Type I TAG, $[MH]^+$ or $[AA]^+$ ($=\Sigma[DAG]^+$). "A" was set as the $[MH]^+$ and "B" was set as the $\Sigma[DAG]^+$, so the $[MH]^+/\Sigma[DAG]^+$ ratio was ≤1. If we divorce ourselves from mass spectrometry of TAG, and say that "A" and "B" are not necessarily related to each other, and both can be any values, then it can be seen that Online Resource 1 is a Unit Anti-Simulacrum (UAS; which also constitutes the First Specified Form of an Anti-Simulacrum), compared to Fig. 4. If "B" is any generic value, and B ≤ 1 is Case 2, then it is the UAS to Fig. 4, in which A ≤ 1 is Case 1.

Type II TAG and Nested Simulacra

The simulacrum solution for a Type II TAG can be understood by examining the Type II Critical Limit, shown in Fig. 6 (repeated from Fig. 3 in the UBUS, also given in Fig. 2 in the BUS), which was $1/(1 + ([AA]^+/[AB]^+))$ or $1/(1 + 1/([AA]^+/[AB]^+))$, depending on whether Critical Ratio 2, $([AA]^+/[AB]^+)$, was <1. It can now be revealed that the CL ratios $1/(1 + ([AA]^+/[AB]^+))$ or $1/(1 + 1/([AA]^+/[AB]^+))$ are really two special cases of the $[MH]^+/\Sigma[DAG]^+$ ratio where the 1 in the numerator is $[MH]^+ = 1$ (as shown in Fig. 6), and the denominator is the simulacrum solution for $\Sigma[DAG]^+ = SimSum([AA]^+,[AB]^+)$, $= Sum([AA]^+ + [AB]^+)$. In the CL either $[AA]^+$ or $[AB]^+$ is 1, which dictates the TAG as Case *n*.1 or Case *n*.2 ("*n*" is the Case for CR1, 1 and 2 are the Cases for CR2). When $[AA]^+$ is substituted into the MSS (Fig. 5) for the generic ion $[A]^+$ in Fig. 5, and $[AB]^+$ is substituted in for the generic ion $[B]^+$, the MSS solution gives one Case 1 solution and one Case 2 solution that can be simplified by cancelling out and ignoring the 1 outside the parentheses, and which use the Critical Ratio constructed for the BUS and UBUS, $[AA]^+/[AB]^+$. The Case 1 solution from Fig. 5 is $(1 + ([AA]^+/[AB]^+))$ and the Case 2 solution is $(1 + 1/([AA]^+/[AB]^+))$. Thus, the simulacrum solution for the $\Sigma[DAG]^+ = SimSum([AA]^+,[AB]^+)$, $= Sum([AA]^+ + [AB]^+)$ is Case 1: $(1 + ([AA]^+/[AB]^+))$ or Case 2: $(1 + 1/([AA]^+/[AB]^+))$, and these two simulacrum solutions are the denominators of the Critical Limit for a Type II TAG. So the special-case $[MH]^+/\Sigma[DAG]^+$ ratio becomes $1/(1 + ([AA]^+/[AB]^+))$ or $1/(1 + 1/([AA]^+/[AB]^+))$, which is the CL. As Scheme 2 shows, when the actual (observed) CR1 is compared to the CL, the Case classification for CR1 is accomplished.

For example, Table 1 shows that LLnL has a CR2 of 0.4793, which is close to the statistically expected value of 1/2 for $[LL]^+/[LLn]^+$. Case *n*.1 in Fig. 6 shows a pretty good representation of $[AA]^+$ being about ½ of $[AB]^+$. To see if the $[MH]^+$ or the $[AB]^+$ is the base peak, while still maintaining the real CR2 of 0.4793, we set $[MH]^+$ equal to 1 and $[AB]^+$ equal to 1, and construct the idealized $1/(1 + ([AA]^+/[AB]^+))$ for Case *n*.1 as $1/(1 + 0.4793) = 0.6760$. Then we compare the observed CR1 to the idealized CR1, and if the observed $[MH]^+/\Sigma[DAG]^+$ were to be less than $1/(1 + 0.4793)$, then the $[MH]^+$ in the numerator must not really be one, it must be <1, so it is not the base peak, and the $[AB]^+$ must be the ion that is 1, and CR1 is Case 1; whereas if the observed $[MH]^+/\Sigma[DAG]^+$ is greater than $1/(1 + 0.4793)$, then the $[AB]^+$ in the denominator must not really be one, it must be <1, so the $[MH]^+$ is the base peak. For LLnL, CR1 is 3.3643, which is ≫0.6760, so the $[MH]^+$ is the base peak. This means the idealized $(1 + ([AA]^+/[AB]^+)$ must be scaled down so that it equals the observed $\Sigma[DAG]^+$ and gives the observed CR1.

Based on the considerations above, it can be seen that the denominator of CR1 is the simulacrum solution for CR2, so CR2 is nested into the denominator of CR1. We can now examine Scheme 2 with new understanding. The Case 1.*n* solutions have either $[AA]^+$ or $[AB]^+$ set to 1, and the other $[DAG]^+$ is CR2 or 1/CR2, and together, these constitute the $\Sigma[DAG]^+ = (1 + ([AA]^+/[AB]^+))$ or $(1 + 1/([AA]^+/[AB]^+))$. Once the $\Sigma[DAG]^+$ is known, it is multiplied times the $[MH]^+/\Sigma[DAG]^+$

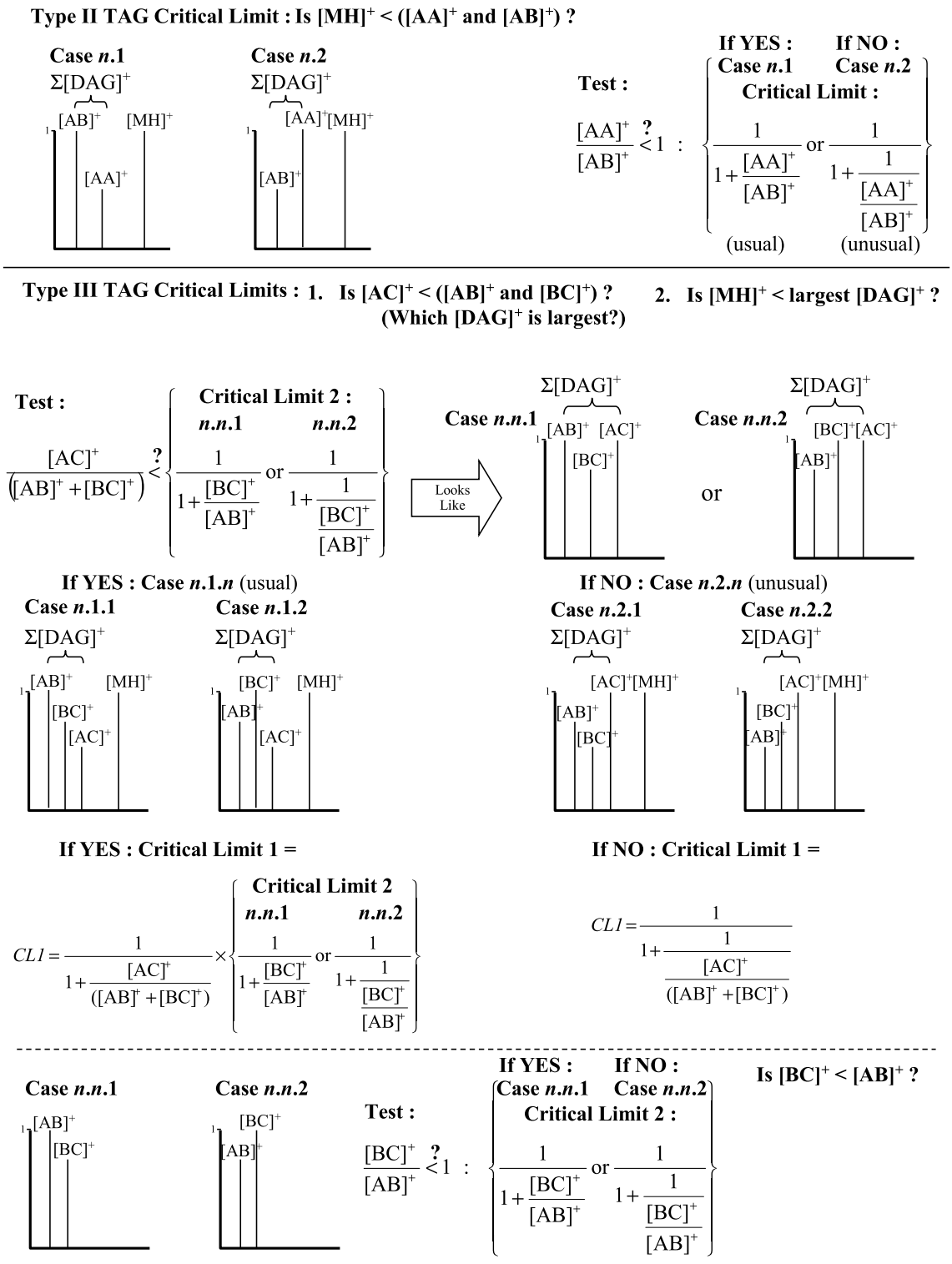
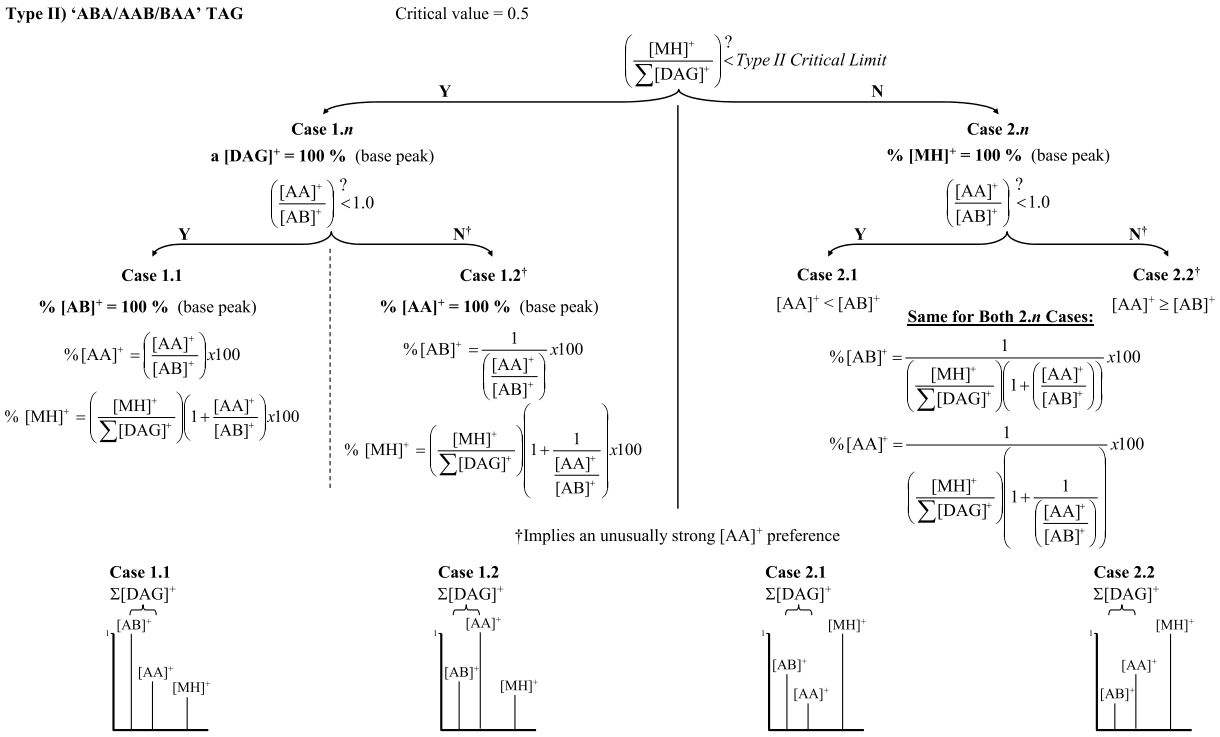


Fig. 6 Idealized representations of mass spectra and equations used to calculate Critical Limits for Type II and Type III TAG

ratio in Scheme 2, and the $\Sigma[DAG]^+$ cancels out yielding the $[MH]^+$. For Case 1.1, the sum of all ions ($[AB]^+$+$[AA]^+$+$[MH]^+$) then becomes: 1 + ($[AA]^+$/$[AB]^+$) + ($[MH]^+$/$\Sigma[DAG]^+$)(1 + ($[AA]^+$/$[AB]^+$)), which rearranges and simplifies to (1 + ($[MH]^+$/$\Sigma[DAG]^+$)) x(1 + ($[AA]^+$/$[AB]^+$)), or (1 + CR1)x(1 + CR2), which is the US for CR1 times the US for CR2. For instance, OLO in Table 1 has CR1 = 0.4652 and CR2 = 0.2931;



Scheme 2 Equations to calculate the relative abundances of the $[MH]^+$ ion and $[DAG]^+$ fragment ions for Type II TAGs using two Critical Ratios from APCI-MS data (Table 1), $[MH]^+/\Sigma[DAG]^+$ and $[AA]^+/[AB]^+$. The Critical Limit for a Type II TAG is given in Fig. 6

CR2 is Case *n*.1, and the CL is 1/(1 + 0.2931) = 0.7733. CR1 < CL, so OLO is Case 1.1, and from Scheme 2 the $[MH]^+$ = CR1x(1 + CR2) = 0.6016, or 60.16 %, $[AB]^+$ = 1, or 100 %, and $[AA]^+$ = 0.2931, or 29.31 %. The sum of all ions, $[AB]^+ + [AA]^+ + [MH]^+$, then is (1 + CR1)x(1 + CR2), or 1.4652 × 1.2931 = 1.8947, or 189.47 %.

Analogously, the sum of all ions ($[AA]^+ + [AB]^+ + [MH]^+$) for Case 1.2 becomes: 1 + 1/($[AA]^+/[AB]^+$) + ($[MH]^+/\Sigma[DAG]^+$)x(1 + 1/($[AA]^+/[AB]^+$)), which rearranges and simplifies to (1 + ($[MH]^+/\Sigma[DAG]^+$))x(1 + 1/($[AA]^+/[AB]^+$)), or (1 + CR1)x(1 + 1/CR2), which is the US for CR1 times the US for 1/CR2. As with Type I TAG, the solutions always contain 1 + ratio or 1 + 1/ratio.

The Case 2.1 and 2.2 solutions are the same as each other, since the $[MH]^+ = 1$ in both cases. Scheme 2 shows that the solutions for $[AA]^+$ and $[AB]^+$ are (1/CR1)(1/(1 + CR2)) and (1/CR1)(1/(1 + (1/CR2))), respectively. This is equivalent to (1/CR1) x CL, which becomes $\Sigma[DAG]^+$ x CL, because when $[MH]^+$=1, 1/CR1 becomes 1/(1/$\Sigma[DAG]^+$), or just $\Sigma[DAG]^+$, and the CL was (1/(1 + CR2)) or (1/(1 + (1/CR2))), as described above. One can think of $\Sigma[DAG]^+$ x CL as scaling down the $[AA]^+$ and $[AB]^+$ in the idealized mass spectrum in the Critical Limit by the observed $\Sigma[DAG]^+$. The Case 2.*n* solutions for $[AB]^+$ and $[AA]^+$ are also simply the inverses of the Case 1.1 and Case 1.2 solutions for the $[MH]^+$, respectively.

The sum of all ions for Case 2.*n* Type II TAG is interesting because of how it simplifies. The sum of $[MH]^+ + [AB]^+ + [AA]^+$ from Scheme 2 is 1 + 1/(CR1x(1 + CR2)) + 1/(CR1x(1 + 1/CR2)), which rearranges to 1 + 1/CR1 x (1/(1 + CR2) + 1/(1 + 1/CR2)). Now, (1/(1 + ratio) + 1/(1 + 1/ratio)) = 1, so the last term in the sum cancels out, and the sum becomes simply 1 + 1/CR1. Note that the relationship (1/(1 + ratio) + 1/(1 + 1/ratio)) = 1 holds true for all MS abundances, and appears to hold true for any real number except 0 and −1 ($\pm\infty$ are not real numbers).

For example, LLO in Table 1 has CR1 = 1.1816 and CR2 = 0.8269; CR2 is Case *n*.1 (Fig. 6), and the CL is 1/(1 + 0.8269) = 0.5474. CR1 ≥ CL, so LLO is Case 2.1, and from Scheme 2 the $[MH]^+ = 1$, or 100 %, $[AB]^+$ = 1/(CR1x(1 + CR2)) = 0.4632, or 46.32 %, and $[AA]^+$ = 1/(CR1x(1 + 1/CR2)) = 0.3831, or 38.31 %. And the sum of all ions is simply 1 + 1/CR1 = 1.8463, or 184.63 %.

Type III TAG

Exactly analogous to the CL for Type II TAG, CL1 and CL2 for Type III TAG are really coded forms of idealized versions of CR1 and CR2. Also, analogous to Type II TAG

is the fact that CR3 is nested into the denominator of CR2 and CR2 is nested into the denominator of CR1, producing a three-level deep nested construct. To save space, Scheme III is not reproduced here, but we will refer to Scheme III in the UBUS. Figure 6 (bottom) shows that Critical Limit 2 is such that $[AC]^+/([AB]^+ + [BC]^+)$ is tested against $1/(1 + ([BC]^+/[AB]^+))$ or $1/(1 + 1/([BC]^+/[AB]^+))$, depending on whether $([BC]^+/[AB]^+) < 1$. The CL2 represents the idealized mass spectra in which $[AC]^+ = 1$ is the numerator of CR2, and either $[BC]^+$ or $[AB]^+$ are set to 1 in the denominator of CR2 (idealized mass spectra are shown to the right of the arrow in Fig. 6). We can see that the denominator, $([AB]^+ + [BC]^+)$, of CR2 equals the simulacrum sum of $[AB]^+$ and $[BC]^+$ if $[BC]^+$ is substituted for the generic ion $[A]^+$ in the MSS in Fig. 5 and $[AB]^+$ is substituted for the generic ion $[B]^+$ in the MSS, so that $[A]^+/[B]^+$ becomes CR3, $[BC]^+/[AB]^+$. Thus, CL2 equals $(1/(1 + CR3))$ or $(1/(1 + (1/CR3)))$, exactly analogous to the CL for a Type II TAG described above.

Because of space constraints, we cannot delve into each solution in Scheme III. But the principles described above repeat themselves over and over, so all solutions are different combinations of the same things, appearing as 1 + ratio or 1 + 1/ratio. Detailed discussion of Case 1.*n.n* solutions for Type III TAG is provided in the Online Resources. To see the patterns behind the nested ratios, it is instructive to substitute 1 in for every abundance in Scheme III, to see how they multiply and cancel out. Of course, the circumstance where every abundance = 1 gives the Critical Values, as stated before.

Discussion

The Critical Ratios for MS of TAG were constructed so that they provided the structural information desired. The new construct called a simulacrum shows all choices, whether constructed or not constructed. For instance, Fig. 2 showed solutions using $[MH]^+/\Sigma[DAG]^+$, but also showed perfectly valid solutions using $\Sigma[DAG]^+/[MH]^+$, which was not constructed. In the BUS and UBUS, CR1 was constructed because $[MH]^+$ had the potential to be zero for saturated TAG. We recently showed that the UBUS applied equally well to ESI–MS and APPI-MS. On another ESI–MS instrument that we recently acquired, TAG can give zero abundance of $[DAG]^+$, unless a small amount of up-front collision energy is applied. Of course, some abundance of $[DAG]^+$ is necessary for identification and quantification of regioisomers. But if ESI–MS on that instrument was used without up-front CID, the $\Sigma[DAG]^+$ could be 0, causing the $[MH]^+/\Sigma[DAG]^+$ to be mathematically undefined. Now that the simulacrum system has been defined, it is possible to see that, in such circumstances, the ratio $\Sigma[DAG]^+/[MH]^+$ could easily be constructed instead, and the solutions would simply contain the inverse of CR1.

Similarly, the $[BC]^+/[AB]^+$ ratio was constructed somewhat arbitrarily, since no trends had been reported to distinguish $[AB]^+$ from $[BC]^+$, prior to publication of the UBUS. Thus, the $[AB]^+/[BC]^+$ ratio could easily have been constructed, and the simulacra indicate that the solutions would be exactly the inverse of the solutions obtained from CR3 as constructed. Thus, a simulacrum shows all possible choices, whether constructed or not constructed, observed or not observed, chosen or not chosen.

This principle is even more apparent in the UIS, S-Fig. 3. In mathematics, our definition of zero is such that $0 < 1$. Nevertheless, a simulacrum of 0 and 1 gives Case 2 solutions, even though these are never observed in reality. For constants greater than 1, such as π, Case 1 is never observed. Nevertheless, a simulacrum always shows all possible solutions whether observed or unobserved, real or unreal, defined or undefined.

The UBUS, which employs the simplified solutions from the MSS, has been shown to apply to APCI-MS of several types of lipids, including TAG, DAG, and vitamin D, and to ESI–MS, ESI–MS/MS, and APPI-MS of TAG and DAG. The simulacrum system described here could readily be applied to other classes of lipids, such as phospholipids, based on Critical Ratios constructed to provide structural information for those. For instance, Hsu and Turk [22] showed that the loss of acyl fragments from phosphatidylethanolamine [PtdEtn, or glycerophosphoethanolamine (GPE) below] provided information about their positions on the phosphoglycerol backbone: "The preferential formations of $R_2CO_2^- > R_1CO_2^-$, and of $[M-H-R'_2CH=C=O]^- > [M-H-R'_1CH=C=O]^-$ are attributed to the findings that charge-driven processes are sterically more favorable at *sn*-2.", and that "These features of tandem spectra readily identify and locate the fatty acid substituents of GPE in the glycerol backbone." Thus, it would be logical to construct the Critical Ratios $[R_1CO_2]^-/[R_2CO_2]^-$ and $[M-H-R'_1CH=C=O]^-/[M-H-R'_2CH=C=O]^-$, as well as $[M-H-R_1CO_2]^-/[M-H-R_2CO_2]^-$ and $[M-H]^-/\Sigma[Frag]^-$ to describe the regioisomers and other structural characteristics of PtdEtn (these were constructed to be Case 1 by default, based on Fig. 1 in [22]). As seen in the BUS and UBUS, one degree of freedom is gained by using Critical Ratios instead of raw abundances (one ratio allows two abundances to be calculated, two ratios provide three abundances, etc.), so there is a diminishing return on the rate of data set compression as the number of ions of interest increases. But since the Critical Ratios provide more structural information than the raw abundances, the increased directly-accessible information may be desired more than the accompanying data compression. To reproduce the 7 ions mentioned above for PtdEtn, 6 ratios would be required. In addition to the four ratios shown,

two more ratios relating the three sets of fragments would be needed, such as ($[M-H-R'_1CH=C=O]^- + [M- H-R'_2CH=C=O]^-$)/($[M-H-R_1CO_2]^- + [M-H-R_2CO_2]^-$), the ratio of the sum of fragments formed by loss of the ketene versus those formed by the loss of the acyl moiety, and ($[R_1CO_2]^- + [R_2CO_2]^-$)/($[M-H-R'_1CH=C=O]^- + [M-H-R'_2CH=C=O]^- + [M-H-R_1CO_2]^- + [M-H-R_2CO_2]^-$), the ratio of the carboxyl anions to fragments formed by loss of the FA chains as carboxyl groups or ketenes. Of course, these are not the only ratios that could be constructed. The first four were chosen because they provide desired structural information about PtdEtn regioisomers, based on literature precedent, and the last two were chosen (constructed) because they are nested to contain the simulacrum sums of the other ratios. For the last two ratios, any ratios that relate one or more values in one preceding ratio to one or more values in another preceding ratio is enough to link the ratios together so that the mass spectrum could be reconstructed, and a compressed data set would be produced. The above ratio examples reproduce only the seven largest and most structurally informative ions from Ref. [22] Fig. 1. For complex mass spectra, such as those produced by atmospheric pressure ionization (API; i.e., APCI, APPI, or ESI) MS/MS using high collision energy, it may be most beneficial to use Critical Ratios to extract desired structural information from only a few of the ions, and to construct a simulacrum solution to reproduce only the subset of ions of interest from those Critical Ratios.

While the simulacrum system, and specifically the US and MSS, is ideally suited to MS, because it is a 1-based system (100 % = 1), it can also be seen to be more widely applicable to any name, number, symbol, or defined entity. This point is exemplified in the Infinity Unit Simulacrum given in Online Resource 5, which is always Case 2.

Infinitely Nested Unit Simulacra

For Type I TAG, the simulacrum sum of all ions, $[MH]^+$ and the one $[DAG]^+$, was (1 + ratio) or (1 + 1/ratio). It should be mentioned that "1 + something" equals "something + 1" (i.e., the commutative property of addition), and that adding 1 to something can be called incrementing that something. Therefore, since 1 + A = A +1, the Unit Simulacrum can be referred to as the First Increment (FI), A + 1. This is in contrast to the FI in the denominator of a ratio, which is the First Decrement (FD), or First Deconstruction, A/(1 + 1). For example, the FI of 1 is 1 + 1=2, and the FD of 1 is 1/(1 + 1) = ½. This is pointed out because the $[MH]^+/\Sigma[DAG]^+$ for Type II TAG gives a ratio of 1/(1 + 1) for the Critical Value, as shown in Fig. 2 of the BUS and UBUS, which is the same as the FD. The importance of this simple idea will be shown in two applications of the US below.

The idea of the First Deconstruction is important because it keeps showing up in the special case of Critical Values, which occurred when all abundances were set equal to 1. And if we extrapolate the three-level deep nesting of the simulacrum solution for MS of TAG to additional nested solutions, we can actually arrive at a solution for Fibonacci ratios (ratios of consecutive Fibonacci numbers). In Fig. 6, the *n.n*.1 CL2 for $[AC]^+/([AB]^+ + [BC]^+)$ is 1/(1 + (1/1)) when $([BC]^+/[AB]^+)$ = (1/1) (at the CV). As mentioned in the Results section, the denominator of CR2 is the simulacrum sum of CR3. Compare this to the first part of Type III CL1 in Fig. 6, which is $1/(1 + ([AC]^+/([AB]^+ + [BC]^+)))$. When we substitute 1/(1 + (1/1)) (at the CV for CR2), which equals 1/2, into the first part of CL1 for Case *n*.1.*n*, we get 1/(1 + (1/(1 + (1/1)))), which equals 2/3. If we keep deconstructing every (1/1) by substituting a Unit Deconstruction, 1/(1 + 1) = 1/(1 + (1/1)), in for every terminal (1/1), we get a series of ratios: 1/(1 + (1/(1 + (1/(1 + (1/1)))))), which equals 3/5; 1/(1 + (1/(1 + (1/(1 + (1/(1 + (1/1)))))))), which equals 5/8, etc. The ratios 1/2, 2/3, 3/5, 5/8, etc. are lesser Fibonacci ratios (<1) that, if extended infinitely, form an infinite series, called a continued fraction, Online Resource 6, that converges to the value of $1/\phi$, where ϕ is the well-known Phi Ratio, also called the Golden Ratio, Golden Mean, divine proportion, or Golden Section. When we make the Unit Simulacrum (1 + ratio) out of this nested set of Unit Deconstructions, we get: 1 + 1/(1 + (1/1)), which equals 3/2, 1 + 1/(1 + (1/(1 + (1/(1 + (1/1)))))), which equals 5/3; 1 + 1/(1 + (1/(1 + (1/(1 + (1/(1 + (1/1)))))))), which equals 8/5, etc. The ratios 3/2, 5/3, 8/5, etc. are greater Fibonacci ratios (>1) that, if extended infinitely, S-Fig. 6, form a continued fraction that converges to ϕ.

Since this brings us to the ϕ ratio, it is interesting to note that the ϕ ratio is the only number found so far that is its own Unit Simulacrum inverse, which is to say the $\phi = 1 + 1/\phi$. Furthermore, this can be deconstructed, $(1/(1 + (1/\phi)))$, once, twice, or infinite times to still produce ϕ, as shown in Online Resource 7.

The point of the discussion above is to demonstrate that the simulacrum system applies not only to APCI-MS, ESI–MS, MS/MS, and APPI-MS of TAG, DAG, and vitamin D, but also applies to MS of phospholipids, as well as other lipids, and is applicable to other types of MS, and further applies to other areas unrelated to mass spectrometry, such that it even provides solutions for Fibonacci ratios and transcendental numbers such as ϕ.

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Compliance with Ethical Standards

Conflict of interest The author owns the trademarks for the name and symbol of the Unit Simulacrum. The author hereby grants full and unrestricted rights to use the name and symbol of the Unit Simulacrum for all purposes educational, commercial, public, private, and otherwise.

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