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Selectivity comparisons of comprehensive two-dimensional gas chromatography systems through the use of a standardized synthetic mixture☆

Jean-Marie D. Dimandja [a,*], Jeffrey C. Archer [a], Erica L. Bakota [a], Jeffrey D. Bruce [a], John A. Casanova [a], Eric A. Posenecker [a], Anaïs Rodrigues [b], Djulia Bensaada [b], Pierre-Hugues Stefanuto [b], Jean-François Focant [b], Daniel R. Struk [c], Rizky Ilhamsyah [c], Peter J. Hesketh [c], Christina N. Kelly [d], Joseph E. Binkley [d], Giorgia Purcaro [e], Aleksandra Gorska [e], Damien Eggermont [e], Marie Vaccaro [f], Pascal Cardinael [f], Christopher A. Heist [g]

[a] US Food and Drug Administration, Office of Laboratory Operations and Applied Science, College Park, Maryland, USA
[b] University of Liège, Organic and Biological Analytical Chemistry Group, Liège, Belgium
[c] Georgia Institute of Technology, GW Woodruff School of Mechanical Engineering, Atlanta, USA
[d] LECO Corporation, Separation Science Applications Division, St. Joseph, Michigan, USA
[e] University of Liège, Gembloux Agro-Biotech Analytical Chemistry Laboratory, Gembloux, Belgium
[f] Univ Rouen Normandie, FR3038, SMS, UR 3233 F-76000 Rouen, France
[g] Georgia Tech Research Institute, Food Processing Division, Atlanta, USA

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ABSTRACT

Comprehensive two-dimensional gas chromatography (GC × GC) systems can be broadly classified according to three important components: the modulator (cryogenic or flow-based), the column set (normal or reversed orthogonality), and the detector (MS or non-MS). In this work a methodology for the comparison of GC × GC systems has been developed. This method is based on the use of a synthetic mixture of chemicals called the Century Mix, which provides a fingerprint of the relative retention coordinates of the mixture components on each system. These retention coordinates are then normalized through the use of temperature-programmed retention indexing (TPRI), and the comparison of these TPRI values from one system to another can establish the degree to which they are similar. A metric called the retention index correlation (RIC) is introduced to measure the goodness-of-fit between systems. In the first phase of the project, the variation of operating parameters (such as flow rate, temperature programming rate, and modulation period) were investigated to determine their impact on the reproducibility of the TPRI values on a single instrument. In the second phase of the project, Century Mix fingerprints were generated on 11 different GC × GC systems, and pairwise comparisons between thermal and flow-based systems with the same stationary phase combination yielded RIC plot r^2 values above 0.999. Advanced data processing techniques (such as principal component analysis, and dendrogram and heatmap plots) were also used to characterize and classify the bidimensional retention maps as a collective group. The method successfully demonstrates the ability to distinguish between significant differences in systems, and will ultimately be helpful in establishing effective comparisons in larger-scale studies.

1. Introduction

The commercialization of GC × GC instrumentation started in 1994 when the Zoex Corporation started to retrofit existing GC instruments with the novel thermal modulation technology that had recently been patented [1,2]. Thirty years later, there are now over 10 companies that

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* Corresponding author.
E-mail address: Jean-Marie.Dimandja@fda.hhs.gov (J.-M.D. Dimandja).

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Table 1
GC × GC System Comparison Studies.

Principal Investigator (Publication Year)	Modulators Investigated	Column Set Type	Testing Sample(s)	Carbon Range	Flow Rate (mL/min)	Temp. Program Range (°C)	Primary Ramp Rate (°C/min)	Modulation Period (*sec*)	Ref #
P. Marriott (2000)	1) Zoex Sweeper 2) LMCS	BPX5/BPX50	Commercial test mixture	C_{13} - C_{16}	1.0	40 - 160	1.0	5.0	13
A. Lewis (2000)	1) LMCS 2) Stainless steel tubing	BP1/BP50	Air sample containing VOCs	C_5 - C_{13}	2.0	35 - 80	1.0	4.5	14
P. Korytar (2003)	1) LMCS 2) Zoex Sweeper 3) Zoex KT-2001 4) Zoex KT-2003 5) In-house dual jet CO_2 6) In-house single jet CO_2	HP-1/BPX50	3 synthetic test mixtures	C_{11} - C_{30}	1.65	50 - 300	5.0	3.0	15
T. Gorecki (2006)	1) Home-built single jet LN_2 2) Home-built stop flow	VF-1/BP20	Unleaded gasoline	C_4 – C_{12}	N/A	35 - 240	5.0	4.0	18
G. Semard (2011)	1) LECO Quad Jet 2) Agilent FFF	DB-5/BPX50	Light cycle oil	C_9 – C_{23}	1) 0.5 2) 0.2 and 22.0	40 - 330	1.9	11.0	19
C. Duhamel (2015)	1) Dual jet CO_2 2) Agilent FFF 3) Home-built 1-plate FFF 4) Home-built 2-plate FFF 5) RFF with adjustable collection channel	DB-1 HT/BPX50 DB-1 HT/ZB-35 HT	Vacuum gas oil, and 2 synthetic mixtures	C_{10} – C_{50}	1) 1.3 2) 0.14 and 24.0	40 - 400	1.9	20.0	20
J. Krupcik (2016)	1) FFF 2) RFF	DB-5 MS/HP-Innowax Rt-β-Dex/HP-Innowax	Gasoline reformate and essential oil sample	C_8 – C_{12}	0.7 and 23.0	40 – 160	2.0	6.0	16
C. Cordero (2017)	1) Zoex KT-2004 2) RFF	PEG Solgel Wax/OV-1701	Fabric softener and natural extracts test mixture	C_9 – C_{25}	1) 1.8 2) 0.3 and 4.5	70 – 260	6.2	4.0	21
F. Magagna (2018)	1) Zoex KT-2004 2) RFF	PEG Solgel Wax/OV-1701	Cocoa	C_9 – C_{25}	1) 1.5 2) 0.3 and 12.0	40 – 230	3.0	3.0	22
R. Striebich (2018)	1) LECO Quad Jet 2) FFF	DB-5 MS/DB-17 MS	Jet Fuel	C_6 – C_{26}	N/A	N/A	N/A	N/A	23
A. Lelevic (2020)	1) FFF 2) FFF	DB-1/BPX50	Alkane mixture	C_8 – C_{28}	0.15 and 11.0	50 – 325	2.5	5.0	17
I. Aloisi (2020)	1) Zoex ZX2 Loop 2) FFF (7-port wafer chip)	SLB-5 MS/SLB-35 MS	Bio-oil (produced from pyrolysis of coconut fibers)	C_5 – C_{28}	1) 0.41 2) 0.24 and 8.4	50 – 330	6.0	4.0	24
B. Giocastro (2020)	1) Zoex ZX2 Loop 2) FFF (7-port wafer chip)	SLB-5 MS/SLB-35 MS	Fragrance allergens test mixtures	C_9 – C_{22}	1) 0.41 2) 0.24 and 8.4	50 – 330	6.0	4.0	25
H. Boswell (2020)	1) Home-built single stage consumable-free 2) SepSolve Insight FFF	Mega-17 MS/Rxi-1 MS	Motor oil	C_{20} – C_{34}	1) 2.0 2) 0.5 and 21.3	40 - 340	5.0	8.0	26
F. Stilo (2020)	1) Zoex KT-2004 2) RFF	DB-1/OV-1701	Fragrance allergens test mixtures	C_9 – C_{25}	N/A	60 – 280	4.0	5.0	27
H. Boswell (2024)	1) LECO Quad Jet 2) RFF	Rtx-1 MS/Rtx-17 Sil MS	Diesel	C_8 – C_{40}	N/A	N/A	N/A	N/A	28

have marketed and/or currently sell GC × GC instrumentation, which is a testament to the overall steady growth of the technology [2–11]. GC × GC instruments can generally be classified into 2 groups based on the modulation mechanism between the two separation columns: thermal (or cryogenic) modulators and flow-based modulators. Even though thermally-modulated systems are still recognized for providing better overall resolution performance, advances in flow-modulated systems have evolved to the point where they are now considered a viable option in a number of applications, and a recent SciFinder Scholar search of over 200 peer-reviewed publications in the past 18 months shows an 80/20 ratio between the reported GC × GC projects that are accomplished on thermally-modulated vs. flow-modulated systems. The increased interest in the use of flow-modulated GC × GC devices has also been reported by Zanella et al. [12].

Table 1 shows an overview of a number of GC × GC system comparisons that have been investigated to date, which have included thermal vs. thermal systems [13–15], flow vs. flow systems [16,17] and thermal vs. flow systems [18–28]. Evaluations within a class of systems (thermal vs. thermal, flow vs. flow) are fairly straightforward due to the fact that the operational conditions (flow rates, temperature programs,

Table 2
List of GC × GC Systems used in this study.

System #	Manufacturer (Model)	Modulator Type	Primary Column phase and dimensions (length, i.d., film thickness)	Secondary Column phase and dimensions (length/i.d./film thickness)	Column set classification	Modulation Period (seconds)	Location
1	LECO (Peg 4D)	Cryogenic (Quad Jet)	Rxi-5 MS (30m/250μm/0.25μm)	Rxi-17 (2m/180μm/0.18μm)	Normal	8.0	Atlanta USA
2	LECO (Peg 4D)	Cryogenic (Quad Jet)	BPX5 (30m/250μm/0.25μm)	Rxi-17 (2m/250μm/0.25μm)	Normal	5.0	Liège Belgium
3	LECO (BT)	Cryogenic (Peltier)	Rxi-5 MS (30m/250μm/0.25μm)	Rxi-17 (2m/250μm/0.25μm)	Normal	4.0	Liège Belgium
4	SepSolve	Flow (Insight FFF)	BPX5 (20m/180μm/0.18μm)	BPX50 (3m/250μm/0.25μm)	Normal	4.5	Gembloux Belgium
5	LECO (BTX)	Flow (Paradigm)	DB-5 (20m/180μm/0.18μm)	Rxi-17 Sil MS (4m/250μm/0.25μm)	Normal	4.0	St. Joseph USA
6	LECO (Peg 4D)	Cryogenic (Quad Jet)	ZB-5 MS (30m/250μm/0.25μm)	VF-200 (2m/250μm/1.0μm)	Normal	11.0	Atlanta USA
7	LECO (BT)	Cryogenic (Quad Jet)	BPX50 MS (30m/250μm/0.25μm)	Rxi-5 (2m/250μm/0.25μm)	Reversed	4.0	Liège Belgium
8	Zoex	Cryogenic (Loop Jet)	DB-17 MS (15m/250μm/0.25μm)	SLB-5 MS (2.5m/100μm/1.0μm)	Reversed	3.5	Rouen France
9	LECO (Peg 4D)	Cryogenic (Quad Jet)	Rxi-17 MS HT (15m/250μm/0.25μm)	Rxi-1 MS HT (0.8m/150μm/0.15μm)	Reversed	6.0	Gembloux Belgium
10	LECO (Peg 4D)	Cryogenic (Quad Jet)	VF-200 (30m/250μm/0.25μm)	Rtx-17 (2m/250μm/1.0μm)	Hybrid Normal	7.0	Atlanta USA
11	LECO (Peg 4D)	Cryogenic (Quad Jet)	Rtx-17 (30m/250μm/0.25μm)	VF-200 (2m/250μm/1.0μm)	Hybrid Reversed	16.0	Atlanta USA

etc.) are essentially the same, resulting in chromatograms that are on the same time scale in both the primary and secondary dimension. The metrics of comparison include the measurement of the peak widths, peak shapes (peak asymmetries) and the relative retention positions (elution order) of the identified target analytes in the samples tested. Comparisons between thermally-modulated systems and flow-modulated systems are more challenging, because the ratios between the primary and secondary column lengths are significantly different. In thermally-modulated systems, the length of the secondary column is typically one-tenth (or less) of the length of the primary column, and a representative column set for this platform is a 30-meter primary column connected to a 2-meter secondary column. In flow-modulated systems the length of the secondary column is generally much more than one-tenth of the primary column length to accommodate the faster flow rates that are needed for high-throughput operation, and a typical system will have a 20-meter primary column connected to a 5-meter secondary column. The resulting chromatograms can still be compared when using the same sample on both platforms, and samples used for thermal vs. flow studies have been related to the specific interests of the research groups involved – primarily petroleum related products (gasoline, jet fuel, motor oil, diesel) [18,19,23,26,28] and foods/fragrance products (bio-oil from coconut fibers, cocoa, fabric softener allergens) [22,24,25,27]. There is a need for a general-purpose mixture that could be used for all GC × GC system comparison investigations, because it would help standardize the evaluation of the key performance parameters that will ultimately lead to the development of methods that are platform independent.

An interagency agreement between the US Food and Drug Administration (FDA) and the National Institute of Standards and Technology (NIST) is currently supporting the development of a synthetic mixture for the evaluation of GC × GC/MS instrument performance, in recognition of the potential of high-resolution separation science-based technologies to generate information-rich databases for the growing field of nontargeted (NTA) and suspect screening analysis (SSA) [29,30]. The mixture is called the Century Mix, and is a modified version of an earlier test mixture for the characterization of GC × GC stationary phase combinations [31]. The Century Mix is composed of an assortment of chemical probes that were selected to cover a relevant portion of the GC × GC chemical space. The information provided is useful in the classification of GC × GC column sets on the basis of the elution orders of the compounds in the primary and the secondary dimension. The use of several series of homologous series of compounds with different structural functionalities is particularly helpful in the determination of the separation gradient on the secondary column, and preliminary testing of the Century Mix has already yielded results that suggest that column selection in GC × GC should be expanded to stationary phase combinations that have not yet been investigated [32].

The goal of this project is to investigate the use of the Century Mix in a variety of GC × GC systems operated at different instrumental conditions in a manner similar to what Kirchkeszner et al. have recently reported for conventional one-dimensional GC [33]. Temperature programmed retention indexing (TPRI) is of great use in GC as an identification tool to supplement additional information provided by a MS detector, or even in systems using other detectors (such as FID or ECD) [35]. Current retention index databases are in need of new approaches to account for the widely different conditions under which the reported data has been catalogued, particularly since the variability differences are compound specific [34]. Using a mixture with a variety of chemical functions has the advantage of generating results for which an average value of the overall change in relative retention can be calculated while also keeping track of changes that occur with respect to each individual compound. This information will ultimately be useful in interlaboratory efforts where changes in different systems can be accounted for. These standardized databases will also find good use in non-targeted analysis because the results from one laboratory can be used to confirm the results in another laboratory pertaining to the same target analyte.

The experimental approach for this project involved the generation of TPRI values for all Century Mix compounds in two different categories of investigations. 1) an intra-laboratory study that focused on the changes in operating parameters on a single system, and 2) an interlaboratory study that focused on the variations in the results from different instruments using different column sets.

2. Experimental

2.1. Chemicals

A stock solution of the mixture of standards was prepared at one of the participating laboratories. Neat individual compounds were purchased from MilliporeSigma (St. Louis, MO). The average concentration of the individual compounds in the stock solution was 500 μg/mL in dichloromethane solvent. This stock solution was aliquoted into 10 mL

amber glass vials and stored in a -80 °C freezer. A set of "confirmation" mixtures were also prepared to confirm the identity of target analytes when necessary. Each homologous series in the Century Mix was prepared separately (11 groups), and the remaining compounds were divided into 5 groups of 4 compounds each, which were assembled to avoid co-elution of any analytes in a given submixture. These confirmation mixtures are important when using systems with non-MS detectors or operating conditions that result in elution patterns that are challenging to make identification determinations due to wraparound and/or co-elution of target analytes.

2.2. 1D GC analyses

Twelve different stationary phases were used to provide 1D GC TPRI information for use in this project. These stationary phases were from Agilent (DB-5, DB-17, DB-1701, DB-35, DB-Wax, DB-EUPAH, and VF-200), Restek (Rxi-1 and Rtx-624), Phenomenex (ZB-50), and Sigma-Aldrich (SP-2330). All columns were 30-meters long, with a 250 μm i.d. and a 0.25 μm film thickness, except for Rtx-624, which was a 60-meter column. An additional DB-5 (60-meter length) was also used to test the impact of column length on retention indexing.

These analyses were performed on an Agilent 7890 GC-FID system (Agilent Technologies, Santa Clara, CA, USA). The flow rate was 1.0 mL/min (helium carrier gas, 99.999% purity), and the inlet temperature was 200 °C. The temperature program started at 40 °C (2-minute hold) with a 5 °C/min ramp to a final temperature of 280 °C (10-minute hold) except for VF-200, SP-2330, and DB-Wax, where the final temperature was 260 °C. The injection volume was 1.0 μL (with a 10:1 split).

2.3. GC × GC analyses

The first set of experiments focused on variation of operating parameters on a single cryogenic system (System 1 in Table 2). The following parameters were changed: flow rate (between 0.5 mL/min and 2.0 mL/min), temperature programming rate (between 2 °C/min and 20 °C/min), modulation period (between 1 s and 16 s), data acquisition rate (between 20 Hz and 200 Hz). In each of these experiments, only the parameter of interest was changed while other operating conditions were kept unchanged for that set of runs in order to properly assess the impact of the parameter of interest on overall changes to the relative retention of the target analytes in the Century Mix. The final set of runs involved a set of replicate runs on different days to study the reproducibility of the system's results at a given set of "standard" operating conditions (flow rate: 1.2 mL/min; temperature programming rate: 5 °C/min; modulation period: 8 s; and data acquisition rate: 100 Hz).

In the second set of experiments, the Century Mix was analyzed on 11 different GC × GC systems in 5 different locations in order to compare their performance at the same (or as similar as possible) operating conditions. An overview of these systems is also provided in Table 2 (a more detailed list of the operating conditions is available in Supplemental Table S1). The systems that were assembled presented a reasonable representation of the GC × GC instruments currently in use today: there are 3 different instrument manufacturers (LECO, Zoex, and SepSolve), 2 different types of modulators (cryogenic and flow), 3 different types of column sets (normal, reversed, and hybrid), and 3 types of detectors (quadrupole MS, TOF MS, and FID). The recommendations to the participating laboratories were to operate the instruments at conditions nearing the following: flow rate near 1.0 mL/min for cryogenic systems and 0.5 mL/min in the first dimension of flow systems; injection volume of 1.0 μL (split ratio was left to the discretion of each individual laboratory); Initial temperature of 40 °C and a temperature ramp rate of 5 °C/min (final temperatures were dependent on the maximum allowable operating temperature of the least polar stationary phase in the column set); and acquisition rates of 100 Hz. Run times ranged between 40–60 min, and modulation periods ranged between 3–16 s. Helium gas was the carrier gas in all 11 systems in this study.

2.4. Data processing

Participating laboratories shared the peak tables of the Century Mix analyses that were performed on their instruments, with adjoining information on the operating conditions and column dimensions of each system that was used. The temperature-programmed retention index (TPRI) values were calculated by summing the first and second retention times for each compound, and then using the van Den Dool interpolation calculation [35]:

$$TPRI = (100 \times z) + 100 \times \left(\frac{t_{S,x} - t_{S,z}}{t_{S,z+1} - t_{S,z}}\right)$$

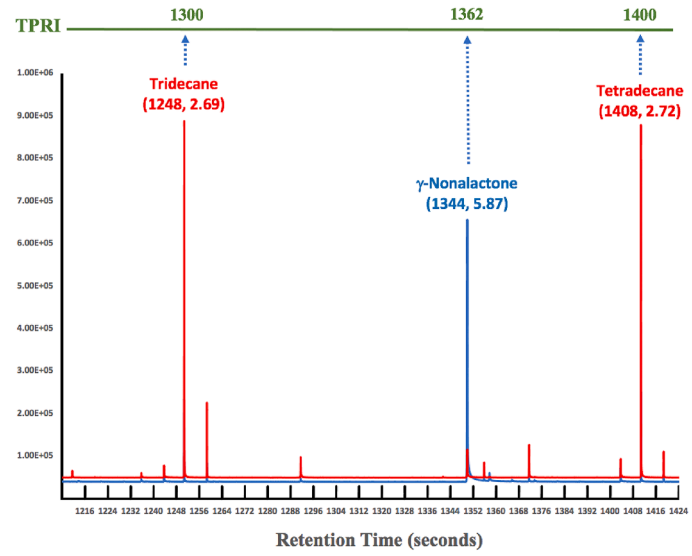
Where $t_{S,x}$ is the summed retention time of the target analyte, $t_{S,z}$ is the summed retention time of the alkane preceding the target compound, $t_{S,z+1}$ is the summed retention time of the alkane following the target compound, and z is the number of carbons of the alkane preceding the target analyte. Evaluations of TPRI information between different systems were done by simply subtracting the value obtained in one run from the other run for every detected analyte. The collection of these differences could then be studied to assess the overall differences between the runs using chemometric tests (such as Principal Component Analysis (PCA), heatmaps, and dendrograms) that were obtained using MetaboAnalyst 5.0 (Xia Lab, McGill University, Montréal, Canada) [36].

3. Results and discussion

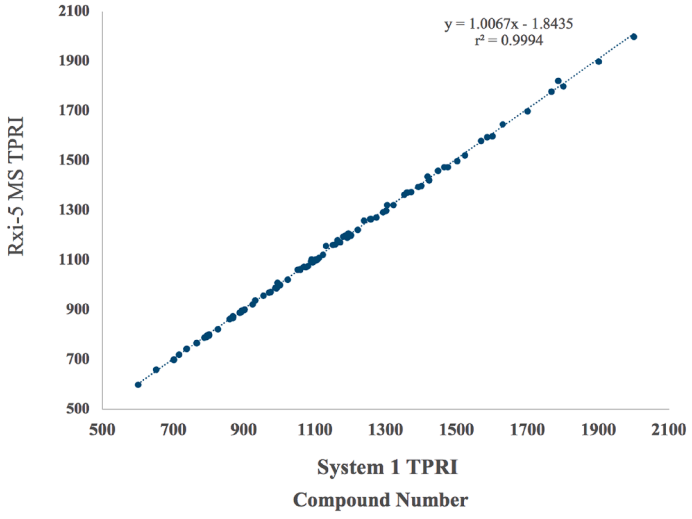
3.1. Development of TPRI comparison metrics

There are currently two approaches for retention indexing in GC × GC. The first approach uses an extension of the retention of saturated alkanes in the second dimension, and interpolates the retention of target compounds with respect to a continuous trace of the alkane compounds (which are called isovolatility curves) in the two-dimensional plane [39]. This method has not been widely applied because it requires a specific set-up and operations that are tedious to implement for routine usage. The use of this method is also limited to the range to which the isovolatility curve will extend in the second dimension, that may preclude the indexing of highly polar compounds. The second approach uses a different set of compounds that is independent from the alkanes in terms of its elution order in the second dimension. Gardner et al. used the aromatic hydrocarbons (benzene, naphthalene, phenanthrene, etc.) which was applied for the profiling of foodborne bacterial fatty acid profiles [40], and Boegelsack et al. later extended this concept for the characterization of ignitable liquid residues in wildfire investigations [41]. Veenas and Haglund used a homologous series of polyethylene glycols in a proof-of-concept project that involved the separation of a synthetic mixture of 72 compounds of interest to the environmental field [42]. The primary challenge of these approaches resides in the need to generate GC × GC chromatograms with no wraparound, because wrapped-around peak retention times in the second dimension lead to retention indices that depend on the modulation period at which they are collected.

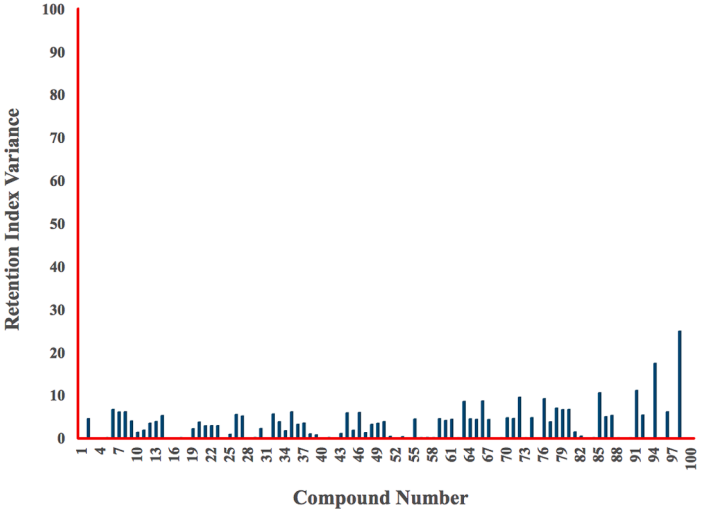
The new indexing approach developed in this work is based on two key observations. The first observation is that GC × GC data is collected as an assembly of fast 1D GC chromatograms, and therefore can be indexed in terms of the raw data and not the processed 2D data. The second observation is that analytes in a GC × GC analysis spend the vast majority of their retention time in the primary dimension, which means that their retention index values will be more closely associated with the primary dimension stationary phase. In System 1 for example, the retention time coordinates for benzene (the first non-alkane eluter) are 160 s in the first dimension and 2.34 s in the second dimension, which means that it spends 98.6% of its retention in the primary column and



A



B



C

Fig. 1. GC × GC TPRI Calculations using System 1 (non-polar/semi-polar) column set. 1A. Raw Chromatographic trace for alkanes bracketing γ-Nonalactone. The selected *m/z* for the alkanes is 57, and for γ-Nonalactone is 85. The y-axis is signal intensity (in arbitrary units). 1B Retention index correlation (RIC) plot between System 1 and a non-polar 1D GC stationary phase. The TPRI for both systems were obtained at the same temperature programmed conditions. 1C. Retention index variance plot between System 1 and the non-polar 1D GC TPRI. Compounds numbers correspond to the elution order listed in Table 3.

Table 3
Comparison of TPRI values between a GC × GC system and its 1D primary column.

	Compound Name	CAS Number	Signature *m/z*	Compound Class	TPRI GCxGC (Rxi-5/Rxi-17)	TPRI 1D GC (DB-5)	ΔI
1	Hexane	110–54–3	57	Alkane	600	600	0
2	Benzene	71–43–2	78	Aromatic Hydrocarbon	651	657	6
3	Heptane	142–82–5	57	Alkane	700	700	0
4	2-Pentanol	6032–29–7	45	Secondary Alcohol	700	704	4
5	Propyl acetate	109–60–4	61	Alkyl Acetate	712	719	7
6	Butanoic acid, methyl ester	623–42–7	74	Fatty Acid Methyl Ester	715	721	6
7	Pyridine	110–86–1	79	Mc Reynolds Compound	737	741	4
8	1-Nitropropane	108–03–2	43	Mc Reynolds Compound	737	744	7
9	1-Pentanol	71–41–0	70	Primary Alcohol	765	766	1
10	Toluene	108–88–3	91	Alkyl Benzene	765	765	0
11	2-Hexanone	591–78–6	58	Ketone	787	789	2
12	1-Octene	111–66–0	56	Alkene	793	791	2
13	2,3-Butanediol	513–85–9	45	Grob Mix Compound	794	804	10
14	Octane	111–65–9	57	Alkane	800	800	0
15	3-Hexanol	623–37–0	59	Miscellaneous	800	801	1
16	2-Hexanol	626–93–7	45	Secondary Alcohol	800	804	4
17	Butyl acetate	123–86–4	61	Alkyl Acetate	814	821	7
18	Butyric acid	107–92×6	60	Carboxylic Acid	824	837	13
19	Pentanoic acid, methyl ester	624–24–8	74	Fatty Acid Methyl Ester	824	824	0
20	Ethylbenzene	100–41–4	91	Alkyl Benzene	858	861	3
21	1-Hexanol	111–27–3	70	Primary Alcohol	867	870	3
22	m-Xylene	108–38–3	91	Xylene isomer	867	871	4
23	p-Xylene	106–42–3	91	Xylene isomer	867	875	8
24	2-Heptanone	110–43–0	58	Ketone	887	890	3
25	1-Nonene	124–11–8	56	Alkene	891	892	1
26	o-Xylene	95–47–6	91	Xylene isomer	891	895	4
27	Nonane	111–84–2	57	Alkane	900	900	0
28	2-Heptanol	543–49–7	45	Secondary Alcohol	900	904	4
29	Pentyl acetate	628–63–7	61	Alkyl Acetate	912	919	7
30	Hexanoic acid, methyl ester	106–70–7	74	Fatty Acid Methyl Ester	922	924	2
31	α-Pinene	7785–26–4	93	Miscellaneous	931	936	5
32	Pentanoic acid	109–52–4	61	Carboxylic Acid	933	949	16
33	Propylbenzene	103–65–1	91	Alkyl Benzene	953	954	1
34	1-Heptanol	111–70–6	70	Primary Alcohol	970	972	2
35	β-Pinene	127–91–3	93	Miscellaneous	974	975	1
36	2-Octanone	111–13–7	58	Ketone	988	991	3
37	Phenol	108–95–2	94	Miscellaneous	990	981	9
38	1-Decene	872–05–9	56	Alkene	991	993	2
39	Hexanoic acid	142–62–1	60	Carboxylic Acid	993	1031	38
40	Decane	124–18–5	57	Alkane	1000	1000	0
41	2-Octanol	123–96–6	45	Secondary Alcohol	1001	1005	4
42	Hexyl acetate	142–92–7	61	Alkyl Acetate	1010	1020	10
43	Heptanoic acid, methyl ester	106–73–0	74	Fatty Acid Methyl Ester	1022	1024	2
44	γ-Hexalactone	695–06–7	85	Lactone	1050	1060	10
45	γ-Terpinene	99–85–4	93	Miscellaneous	1057	1062	5
46	Butylbenzene	104–51–8	91	Alkyl Benzene	1057	1058	1
47	Acetophenone	98–86–2	105	Miscellaneous	1067	1072	5
48	1-Octanol	111–87–5	70	Primary Alcohol	1074	1073	1
49	m-Cresol	108–39–4	108	Miscellaneous	1080	1081	1
50	Guaiacol	90–05–1	109	Miscellaneous	1089	1094	5
51	Heptanoic acid	111–14–8	60	Carboxylic Acid	1089	1123	34
52	1-Undecene	821–95–4	61	Alkene	1091	1093	2
53	2-Nonanone	821–55–6	58	Ketone	1092	1092	0
54	Undecane	1120–21–4	57	Alkane	1100	1100	0
55	Linalool	78–70–6	93	Miscellaneous	1101	1106	5
56	2-Nonanol	628–99–9	45	Secondary Alcohol	1105	1105	0
57	2,6-Dimethylphenol	576–26–1	122	Grob Mix Compound	1110	1106	4
58	Octanoic acid, methyl ester	111–11–5	74	Fatty Acid Methyl Ester	1122	1124	2
59	2-Ethyl hexanoic acid	149–57–5	88	Grob Mix Compound	1131	1177	46
60	γ-Heptalactone	105–21–5	85	Lactone	1150	1160	10
61	Pentylbenzene	538–68–1	91	Alkyl Benzene	1163	1175	12
62	2,6-Dimethylaniline	87–62–7	121	Grob Mix Compound	1163	1175	12
63	1-Nonanol	143–08–8	70	Primary Alcohol	1170	1173	3
64	Octanoic acid	124–07–2	60	Carboxylic Acid	1179	1198	19
65	Naphthalene	91–20–3	128	Aromatic Hydrocarbon	1184	1188	4
66	2-Decanone	693–54–9	58	Ketone	1191	1193	2
67	Methyl salicylate	119–36–8	120	Miscellaneous	1193	1202	9
68	Dodecane	112–40–3	57	Alkane	1200	1200	0
69	2-Decanol	1120–06–5	45	Secondary Alcohol	1200	1205	5
70	Nonanoic acid, methyl ester	1731–84–6	74	Fatty Acid Methyl Ester	1220	1223	3
71	γ-Octalactone	104–50–7	85	Lactone	1255	1265	10
72	Hexylbenzene	1077–16–3	91	Alkyl Benzene	1258	1263	5
73	1-Decanol	112–30–1	70	Primary Alcohol	1272	1274	2

(*continued on next page*)

Table 3 (*continued*)

	Compound Name	CAS Number	Signature *m/z*	Compound Class	TPRI GCxGC (Rxi-5/Rxi-17)	TPRI 1D GC (DB-5)	ΔI
74	2-Undecanone	112–12–9	58	Ketone	1291	1298	7
75	Tridecane	629–50–5	57	Alkane	1300	1300	0
76	2-Aminoacetophenone	551–93–9	120	Miscellaneous	1303	1315	12
77	Decanoic acid, methyl ester	110–42–9	74	Fatty Acid Methyl Ester	1321	1323	2
78	Eugenol	97–53–0	164	Miscellaneous	1352	1362	10
79	γ-Nonalactone	104–61–0	85	Lactone	1358	1369	11
80	Heptylbenzene	1078–71–3	91	Alkyl Benzene	1361	1367	6
81	1-Undecanol	112–42–5	70	Primary Alcohol	1371	1375	4
82	2-Dodecanone	6175–49–1	58	Ketone	1391	1395	4
83	Tetradecane	629–59–4	57	Alkane	1400	1400	0
84	Dicyclohexylamine	101–83–7	138	Grob Mix Compound	1417	1427	10
85	Undecanoic acid, methyl ester	1731–86–8	74	Fatty Acid Methyl Ester	1422	1423	1
86	Dimethyl phthalate	131–11–3	163	Phthalate	1446	1455	9
87	Octylbenzene	2189–60–8	91	Alkyl Benzene	1464	1470	6
88	1-Dodecanol	112–53–8	70	Primary Alcohol	1474	1476	2
89	Pentadecane	629–62–9	57	Alkane	1500	1500	0
90	Dodecanoic acid, methyl ester	111–82–0	74	Fatty Acid Methyl Ester	1523	1523	0
91	Nonylbenzene	1081–77–2	91	Alkyl Benzene	1567	1575	8
92	Diethyl phthalate	84–66–2	149	Phthalate	1586	1593	7
93	Hexadecane	544–73–3	57	Alkane	1600	1600	0
94	Benzophenone	119–61–9	105	Miscellaneous	1629	1633	4
95	Heptadecane	629–78–7	57	Alkane	1700	1700	0
96	Dipropyl phthalate	131–16–8	149	Phthalate	1767	1774	7
97	Phenanthrene	85–01–8	178	Aromatic Hydrocarbon	1786	1791	5
98	Octadecane	593–45–3	57	Alkane	1800	1800	0
99	Nonadecane	629–92–5	57	Alkane	1900	1900	0
100	Eicosane	112–95–8	57	Alkane	2000	2000	0

1.4% in the secondary column. Phenanthrene (the last non-alkane eluter) has retention coordinates of 1888 s and 7.69 s in the primary and secondary columns respectively, which translates to 99.6% of its retention spent in the primary column and 0.4% in the secondary column. The sum of primary and secondary retention times therefore represents the retention time (in the raw chromatogram) of each compound at its modulated peak maximum, and these retention times are then indexed with respect to the alkanes that elute before and after it.

Fig. 1A illustrates the process of how the TPRI is calculated using this method. The total ion chromatogram of the raw chromatographic GC × GC trace has been filtered to focus on the compound of interest (γ-Nonalactone, with a signature *m/z* of 85) and the preceding and following alkanes (tridecane and tetradecane, with a signature *m/z* of 57). The peaks chosen for the three compounds correspond to the modulations that produce the largest subpeak in terms of peak area. Tridecane is modulated four times, but its largest peak is located at (1248, 2.69) seconds. Since we are working on the raw data trace, this peak apex has a value equal to the sum of the first and second dimension, which is 1250.69 s. Similarly, the peak apex retention times for γ-nonalactone and tetradecane are 1349.87 s (1344, 5.87) and 1410.72 s (1408, 2.72), respectively. The resulting TPRI value for γ-Nonalactone is 1362. One could expect some variation around this mean value that would be due to the peak apex shifting by one modulation period from one analysis to another, but those types of variations are consistent with the ± 12 RI unit threshold that is typically applied in conventional 1D GC (34).

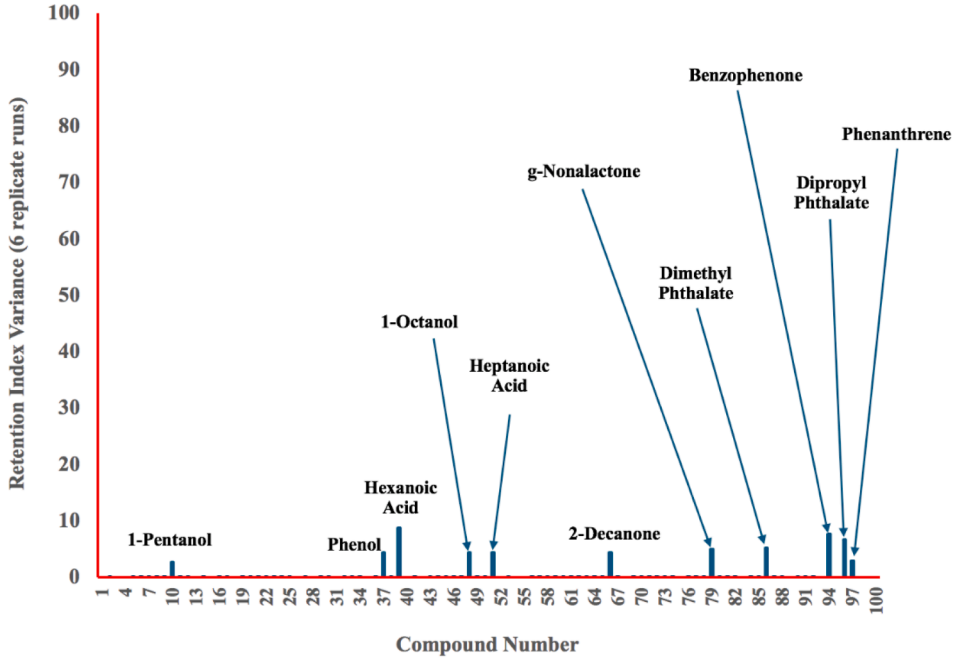
Fig. 1B is a retention index correlation (RIC) plot that compares the TPRI values for System 1 (Rxi-5MS primary column connected to a Rxi-17 secondary column) with the TPRI values obtained on a 1D GC stationary phase that corresponds to the primary column in System 1 (in this case a DB-5 stationary phase). The coefficient of determination (r^2) is of great interest in the RIC plot, because it provides a measure of the similarity between the two compared separation profiles. The r^2 value of 0.9994 suggests a very strong correlation between System 1 and a DB-5 non-polar column. A similar comparison of System 1 with the TPRI of a DB-17 1D GC stationary phase result in an r^2 value of 0.9337, which confirms the stronger retention influence of the primary column on the overall relative retention profile of the analytes in the GC × GC system.

Table 4
RIC plot r^2 values and relative polarity factors (RPF) for GC × GC System (System 1, Table 2) compared to 1D GC Stationary phases.

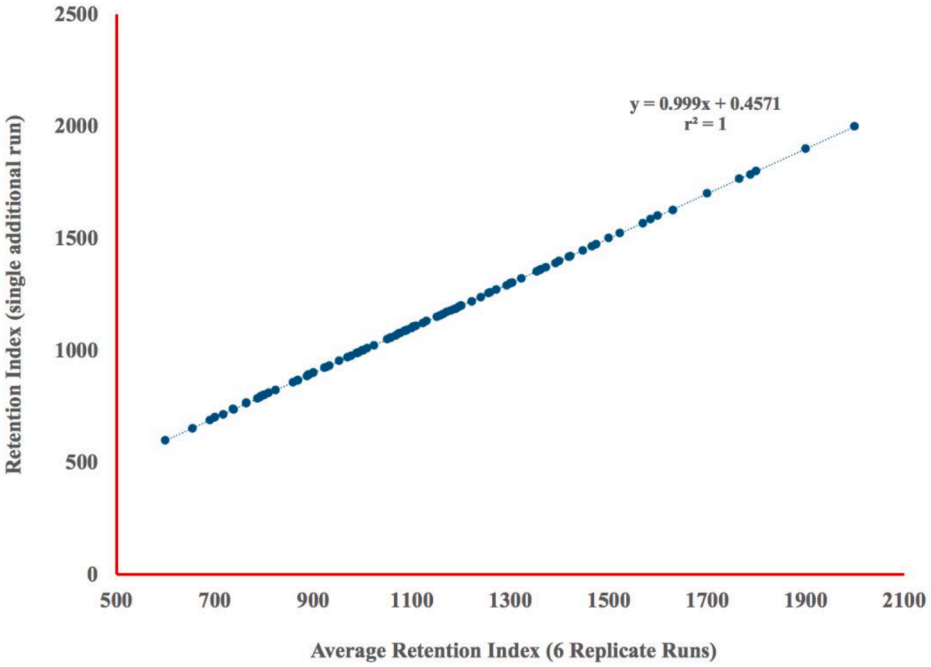
Stationary Phase	Relative Polarity Factor (RPF)	r^2
DB-5 (30 m)	20	0.9994
DB-5 (60 m)	24	0.9994
Rxi-1 (30 m)	92	0.9977
Rtx-624 (60 m)	308	0.9807
DB-35 (30 m)	488	0.9592
DB-1701 (30 m)	489	0.9535
DB-17 (30 m)	664	0.9337
DB-EUPAH (30 m)	672	0.9303
ZB-50 (30 m)	680	0.9297
VF-200 (30 m)	749	0.9039
SP-2330 (30 m)	1350	0.4030
DB-Wax (30 m)	2015	0.5442

Another way to interpret the importance of the RIC plots is to think about the ability to use 1D GC retention index values to predict the relative retention in a GC × GC system.

Fig. 1C is a retention index difference (ΔI) histogram, that accounts for the individual differences (in terms of index units) on a compound by compound basis. This plot helps to visualize which compounds provide a strong match in the RIC plot and which compounds show more variation. The ΔI values are expressed in absolute values in the histogram so that the average and median values only reflect the difference itself and not the direction of the difference (a complete list of these Δis provided in Table 3). The average ΔI of 3 and the median ΔI of 4 indicate that the spread of these values is not skewed. It is important to note that the ΔI of the 15 alkanes in the mixture are not incorporated in these calculations since those values are equal to zero by definition. In Fig. 1C, the compounds that exceed the 12 index unit threshold are all carboxylic acids, and this information is valuable because it helps the investigator in providing different criteria for the confirmation of these compounds on the basis of this knowledge. We have also observed that functional groups tend to behave similarly in terms of their ΔI, and have therefore also generated average ΔI values for each functional group in the Century Mix. The average ΔI for System 1 compared to the DB-5 stationary phase are (in increasing order): alkenes (2 RI units), FAMEs (2 RI units),



A



B

Fig. 2. Reproducibility results for the Century Mix analyses on System 1 (non-polar/semi-polar column set). 2A. Retention index variation plot for the 6 Century Mix replicate runs performed on 2 separate days. The variance was calculated as the difference between the high and low TPRI values obtained during the replicate analyses. 2B RIC plot between the average TPRI of the 6 replicate runs shown in Fig. 2A and the TPRI of a single run performed on a different day on the same System.

primary alcohols (2 RI units), ketones (3 RI units), secondary alcohols (3 RI units), alkylacetates (3 RI units), alkylbenzenes (8 RI units), phthalates (8 RI units), lactones (10 RI units), and carboxylic acids (30 RI units).

In order to place the scope of the RIC plot r^2 values in a greater context, a comparison of the System 1 retention index profile was plotted against a variety of 1D GC stationary phases spanning a wide polarity range. These comparisons are summarized in Table 4, where each individual 1D stationary phase is ranked in terms of how close the analyte TPRI on that column can predict where the TPRI is most likely to

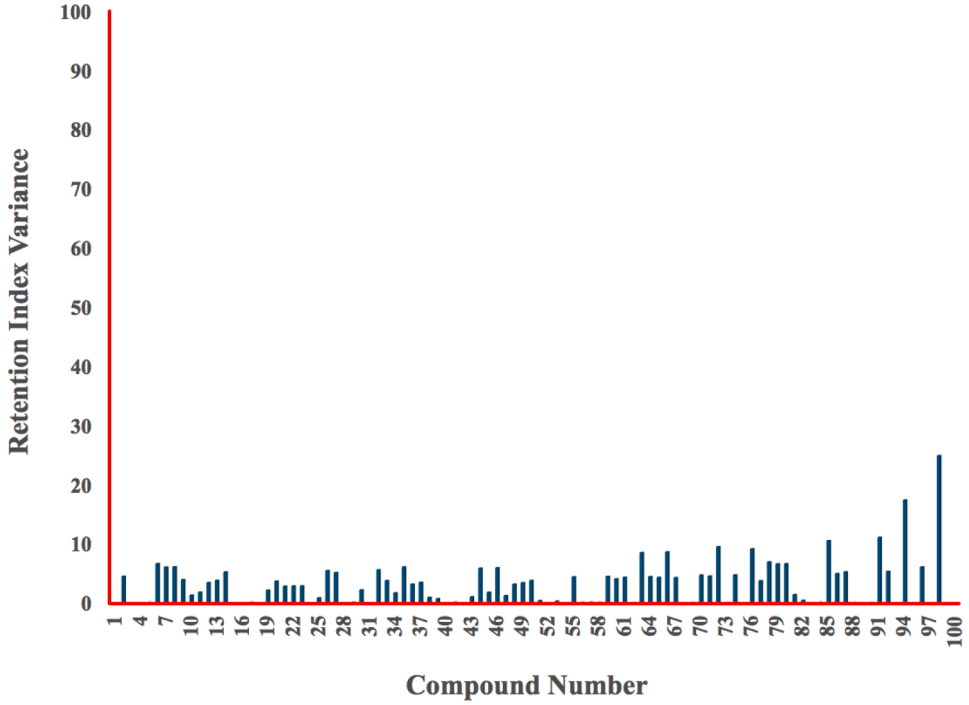


Fig. 3. Retention index variance plot of System 1 (non-polar/semi-polar column set) between flow rates of 0.5 mL/min and 2.0 mL/min.

be found in the GC × GC system. From this set of r^2 values we get a better sense of how to assess the strength of the compared RI information. It appears that $r^2 > 0.999$ indicates a solid match between the 1D stationary phase and the primary column of the GCxGC system. The r^2 values also seem to confirm that column length is not a big factor in the variances of the TPRI, since the 30-meter and 60-meter DB-5 columns show very high similarity to System 1. In addition to the r^2 comparison, another parameter is calculated to provide a measure of the similarity of the systems in terms of overall selectivity of the systems. This parameter, called the relative polarity factor (RPF) is determined on the basis of five compounds (benzene, 1-pentanol, 2-hexanone, 1-nitropropane, and pyridine) that are in the Century Mix. These five compounds correspond to the same probes used in the Rohrschneider-Mc Reynolds polarity measurement, with the exception of 1-pentanol and 2-hexanone that were substituted for 1-butanol and 2-pentanone respectively [43]. The reason for these substitutions is because these two compounds elute before hexane (the lowest alkane marker in the Century Mix), which would make retention indexing impossible on the non-polar stationary phases. The substitution with another primary alcohol and a ketone is done to conserve the role of these probes in providing information on hydrogen bonding (1-pentanol) and dipole-dipole/proton acceptor (2-hexanone) interactions between solutes and the stationary phase. The RPF is thus the sum of the absolute values of the retention index differences between the GCxGC system and the 1D GC column for these five probes, and provides a measure of the relative polarity differences between the two sets of normalized retention data. These two measurements enable the user to classify the overall similarity of a GC × GC system to other gas chromatographic systems, which can be very useful when a given laboratory would like to investigate new or untested column combinations.

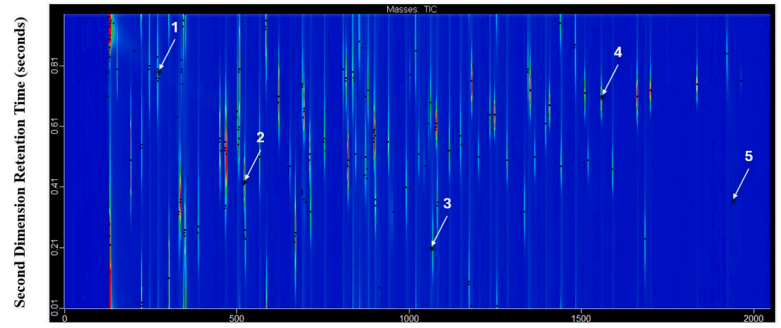
An important distinction should be made between the RPF and the Rohrschneider-McReynolds polarity measurement. In the Rohrschneider-McReynolds system, the ΔIs of each probe are obtained between the retention index of the particular phase when compared to the retention index on a squalane column (which is considered the benchmark of the least polar stationary phase). This measurement gives the value of the absolute polarity of a system. The RPF measurement is an indication of the polarity difference between any two systems, and provides a metric through which comparisons can be made to establish the equivalence of two phases from different manufacturers, or the batch-to-batch differences from a single manufacturer. The impact of other instrument conditions (such as stationary phase film thickness, which was not done in this study) can also be investigated through the use of these measurements.

3.2. *GC × GC intra-system changes*

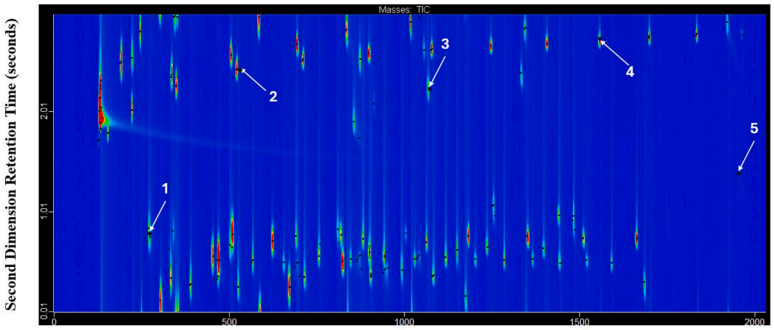
3.2.1. *Reproducibility at standard operating conditions*

The basis of this comparison method is the reproducibility of TPRI results at a given set of operating conditions that would be determined as standard. These conditions were chosen according to what would be deemed reasonable to produce a GC × GC run of adequate resolution and analysis time. The initial temperature (40 °C) was selected because most systems equilibrate fairly rapidly at this temperature and do not require the use of cryogenic fluids to cool the oven. The hold time of 2 min at this initial temperature is done to slow down the more volatile solutes during the time the solvent peak is travelling through the column. The 5 °C/min ramp rate is in line with what most previous system comparison studies have used (see Table 1), and the final temperature is selected not to exceed the maximum allowable operating temperature (MAOT) of the most polar stationary phase in the column set to minimize column bleed. The oven temperature is to be held at the MAOT until the end of the run to allow the elution of all the compounds in the mixture.

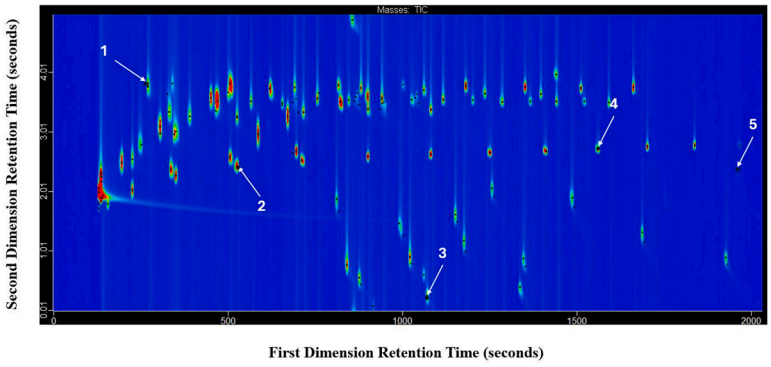
A series of replicate runs (2 sets of triplicate runs on different days) were performed in one of the participating laboratories, and the results of these replicate runs is shown in Fig. 2A. All peaks were manually identified in the chromatogram according to their signature *m/z* ion (provided in Table 3), and confirmed through the peak table information that was the result of the automated software processing. The vast majority of the peaks in the mixture showed less than a 1-unit retention index deviation over the series of replicate runs. For the remaining compounds that showed some deviation, the variability did not exceed 9 RI units (hexanoic acid in Fig. 2A). This variation could be explained by a slight shift (1 modulation period) in the peak apex between runs, and



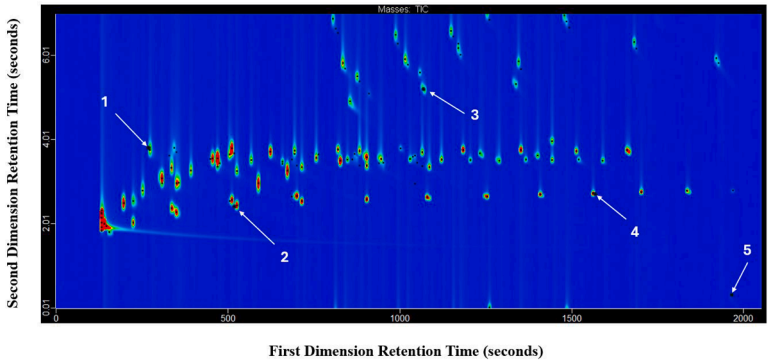
A



B



C



D

Fig. 4. Contour plot chromatograms for GCxGC analyses performed at different modulation periods. System 1 (non-polar/semi-polar column set) was used for all runs in this data set. The 5 compounds identified are: 1) Nonane, 2) Pyridine, 3) Methyl salicylate, 4)Pentadecane, and 5) Phenanthrene. 4A. 1-second modulation period. 4B 3-second modulation period. 4C. 5-second modulation period. 4D 7-second modulation period.

Table 5
TPRI vs modulation period of three selected (early, middle and late eluter) Century Mix Compounds.

	Pyridine				Methyl Salicylate				Phenanthrene			
Modulation Period (*sec*)	RT1 (*sec*)	RT2 (*sec*)	TPRI	Wraparound Factor	RT1 (*sec*)	RT2 (*sec*)	TPRI	Wraparound Factor	RT1 (*sec*)	RT2 (*sec*)	TPRI	Wraparound Factor
1	270	0.76	738	3	1068	0.22	1194	5	1952	0.33	1793	7
2	270	1.77	738	1	1068	1.22	1193	2	1952	1.36	1796	3
3	273	0.76	739	1	1068	2.25	1195	1	1953	1.37	1792	2
4	268	3.79	737	0	1068	1.28	1193	1	1952	3.38	1794	1
5	270	3.77	737	0	1070	0.27	1193	1	1955	2.36	1792	1
6	270	3.77	735	0	1068	5.24	1195	0	1956	1.36	1794	1
7	273	3.76	740	0	1071	5.19	1197	0	1960	0.32	1793	1
8	272	3.77	739	0	1072	5.19	1197	0	1952	7.35	1791	0
9	279	3.72	744	0	1071	5.25	1196	0	1953	7.33	1790	0
10	280	3.65	740	0	1070	5.28	1196	0	1960	7.22	1796	0
11	275	3.77	738	0	1067	5.27	1190	0	1958	7.26	1795	0
12	276	3.76	738	0	1068	5.27	1194	0	1956	7.29	1794	0
13	273	3.78	731	0	1079	5.14	1194	0	1963	7.19	1793	0
14	280	3.73	735	0	1078	5.18	1194	0	1960	7.24	1792	0
15	285	3.7	739	0	1080	5.15	1193	0	1965	7.17	1792	0
16	288	3.69	739	0	1088	5.03	1201	0	1952	7.38	1791	0

primarily involved compounds that contained aromatic rings or analytes with wider peak widths on the primary column stationary phase.

A final confirmation of the reproducibility was done through the use of a single run (on a different day from the previous series of triplicate runs described previously), and the result of this comparison is shown in Fig. 2B The RIC plot r^2 of 1 does not mean that all RI values were identical in the comparison; but it does indicate that the RI differences were very small overall. In fact, there were 43 compounds with 0 RI difference, 23 compounds within 1 RI difference, and 26 compounds within 2 RI units difference. Seven compounds (1-pentanol, 2-decanone, γ-nonalactone, nonylbenzene, dimethyl phthalate, benzophenone, and phenanthrene) were within 3 RI units, and 1 compound (benzene) was within 5 RI units. The average difference of the TPRIs in this run and their corresponding (averaged) values for the replicate runs is 1 RI unit, which is a strong indication of very consistent results at these operating conditions (this averaged difference does not include the 15 alkanes, which by definition will produce a 0 RI difference). The variability of these values may increase in an interlaboratory study due to a host of factors that may include location altitude, instrument manufacturer, slight differences in column dimensions, etc. However, we do not expect these variations to be significantly different from the ones obtained in this work, which is very promising for the establishment of a solid database of information using these standard conditions.

3.2.2. Flow rate

Five different flow rates (0.5, 1.0, 1.2, 1.5, and 2.0 mL/min) were run on the same instrument (System 1 in Table 2) using the standard operating temperature program at a modulation period of 8 s and a data acquisition rate of 100 spectra/*sec*. Fig. 3 is a RI difference histogram between the 0.5 mL/min and the 2.0 mL/min analyses. There are only two compounds (benzophenone, $\Delta I = 17$, and phenanthrene, $\Delta I = 25$) that display variances above the 12 RI units threshold, which strongly suggests that flow rate changes do not significantly impact the relative retention of the compounds in a separation method. It should be noted that an RI histogram generated between 1.0 mL/min and 2.0 mL/min has all compounds listed with ΔIs below 12. Given the fact that the preponderance of GC × GC cryogenic system work is performed at flow rates between 1.0 and 1.5 mL/min, we are prepared to affirm that flow rate selection is not a critical factor in the variation of RI values when comparing results on these systems. More investigations involving the role of column dimensions (particularly stationary film thickness) would be useful to gain a complete understanding of the variability range of these retention indexed values, but the general assertions obtained through this study are valuable in determining the metrics through which the comparisons can be evaluated.

3.2.3. Data acquisition rate

GC × GC systems using fast scanning detection (FID, TOF MS, etc.) are typically operated in the data acquisition rate range between 100–200 Hz, whereas the data acquisition rate range in slower-scanning devices (e.g. single or triple quadrupole MS) is generally between 20–50 Hz. We therefore wanted to document the TPRI results for experiments run at the standard operating procedures while varying the data acquisition rate at 20, 100 and 200 Hz. The average TPRI differences were 1 (at 20 Hz), 2 (at 100 Hz), and 2 (at 200 Hz), with all compounds in the mix below 10 RI units. While data acquisition rate may have an impact on system performance factors such as compound detectability at low concentrations or compound identification (through deconvolution of the MS spectra), it does not change the relative retention values as one might expect. The selection of the data acquisition rate is therefore only dependent on the needs of the user's application.

3.2.4. Modulation period

The modulation period is generally selected in GC × GC to accommodate the elution of the target analytes in the same secondary chromatogram in which they were modulated, in order to avoid peak wraparound. Even though this criterion is desirable to conserve the visibly ordered nature of the bidimensional separation, it cannot always be achieved based on the nature and/or diversity of the analytes co-existing in a given sample matrix. When performing targeted analysis, the ultimate goal is the separation of the compounds of interest, and this goal can be met even if some of the analytes are wrapped around as long as they do not co-elute with other compounds in the chromatogram. For non-targeted analysis, the structure of the chromatogram is very important in visually recognizing new or unknown peaks that may be related to known peaks based on where they are located in the two-dimensional separation space. For the purposes of this project, we wanted to investigate the impact of the modulation period on the relative retention of the compound analytes as expressed through their TPRI. A series of experiments were generated at the standard operating conditions while varying the modulation period in the range between 1 and 16 s.

Fig. 4 shows the contour plots of four selected modulation experiments at 1, 3, 5, and 7 s. Five compounds are identified in these profiles to provide some reference points for comparisons: two alkanes [nonane (1) and pentadecane (4)] help visualize the location of the alkane homologous series line, and three aromatic compounds [pyridine (1), methyl salicylate (3), and phenanthrene (5)] to follow the relative positioning of non-homologous series compounds that elute at the beginning, middle, and end of the chromatographic run. In Fig. 4A (1-second modulation period) the structured separations that are typically

associated with GC × GC separations is not evident due to the fact that every compound in the chromatogram is wrapped around: the two alkanes are wrapped around twice, pyridine is wrapped around 3 times, methyl salicylate is wrapped around 5 times, and phenanthrene is wrapped around 7 times. In spite of the fact that this contour plot is not structured, all 100 analytes are separated and identified by their retention time coordinates. Indexing of the peaks in this chromatogram would be a challenge using the isovolatility method because those traces would be wrapped around throughout the chromatogram. The use of a secondary set of compound markers would also be problematic in this case because those markers would also be wrapped around, and a method to recover the structure of the chromatogram would need to be implemented since the wraparound factor is not the same for all compounds in the chromatogram. The method of using the raw chromatographic trace is advantageous because it is independent of the modulation period, so long as the target compounds are properly identified (the TPRI values for the 3 non-alkane compounds are reported in Table 5). GC × GC/MS separations benefit from the MS library database for the confirmation of the target analytes. GC × GC-FID instruments may require the use of additional confirmation analyses using subsets of standards in order to determine the retention time coordinates in the non-structured two-dimensional contour space.

Fig. 4B is a much more structured chromatogram than Fig. 4A, and displays a significant amount of wraparound. Even though the alkanes are unwrapped, pyridine and methyl salicylate are wrapped around once, and phenanthrene is wrapped around two times. Some GC × GC software programs resolve the wraparound in this contour plot by shifting the retentions of the alkanes to the low end of the second dimension retention time axis. While this approach is valid from the standpoint of creating a more visually pleasing ordered chromatogram, it should be noted that it may not resolve all the wraparound issues. Phenanthrene, which is wrapped around twice, would still be wrapped around once in the shifted contour plot chromatogram. In terms of retention indexing, however, the values of all the compounds are consistent regardless of which modulation period is used (see Table 5 results). Figs. 4C and 4D represent GC × GC chromatograms that most users are more familiar with, in which the wraparound compounds are more visually seen below the early secondary elution line (delineated by the alkanes), and the choice to lengthen the modulation period to unwrap all peaks is a more a matter of preference than necessity.

Table 5 shows that once a peak is unwrapped, its retention time is very consistent. The averaged second dimension retention times (for all modulation periods between 4 and 16 s) is 3.74 ± 0.11 s for pyridine, 5.20 ± 0.17 s for methyl salicylate, and 7.27 ± 0.11 s for phenanthrene. The determination of the true retention time for a compound only requires two separate modulation periods, but its retention indexing only requires one analysis at any modulation period using this method. During the method development phase, the true retention times of the compounds can be determined with longer modulation periods, and then the separation can be optimized at shorter modulation periods with no fear of losing the TPRI values for compound identification. This may be of valuable interest in non-targeted analysis workflows.

The RIC plot r^2 comparing the furthest two analyses in this series of modulation period experiments (1-second and 16-seconds) was 0.9998. The average ΔI for this data set was 3 (with a median ΔI of 2), and all compounds were below a ΔI of 12. All this information indicates that modulation period is not a critical factor in the variation of relative retention, which is an important finding due to the fact that this parameter is (in practice) likely the most variable from one system and/or sample to the next based on the needs of the user.

3.2.5. Temperature programming rate

The dependence of retention index data on temperature has been well studied in past investigations. Very recently, Matyushin and Sholokhova have reported the dependence of retention indices on the column heating rate in the linear temperature programming mode through the use of a large and diverse data set (756 compounds) extracted from the NIST 20 database [37]. In this part of the study we modified the temperature programming ramp through the selection of 5 discrete values in the range between 2 °C/min and 20 °C/min, while keeping all the other parameters the same as in the standard operating protocol. Table 6 is a pairwise comparison chart that contains the RIC plot r^2 values for the temperature programmed analyses conducted in this study. The chart results indicate that r^2 values for 2 °C/min vs.15 °C/min and 2 °C/min vs. 20 °C/min fall below the 0.999 threshold, which would suggest that larger ramp rate differences may result in retention index differences that become too large to deem them equivalent. It does appear that ramp rates within a 10 °C/min range yield relative retentions that are adequately similar.

Table 6
RIC plot r^2 for pairwise comparisons of temperature programming rates for System 1 (Table 2).

	5 °C/min	10 °C/min	15 °C/min	20 °C/min
2 °C/min	0.9997	0.9993	0.9986	0.9983
5 °C/min		0.9997	0.9993	0.9991
10 °C/min			0.9996	0.9994
15 °C/min				0.9993

Fig. 5 presents a retention index variance histogram between TPRI values obtained at 2 °C/min and 20 °C/min, since these two analyses represent the widest range in analysis times for all the testing that was done in this project. The main observation from this comparison is that the variance in retention index values is noticeably lower for aliphatic compounds than it is for aromatic compounds. Of the 47 aliphatic compounds in the Century Mix, only 2 carboxylic acids (heptanoic acid and octanoic acid) have ΔI values above 12 index units, whereas 20 of the 38 aromatic compounds are above the 12 index unit threshold (the 15 alkanes are not counted in the aliphatic group, since their retention index differential is equal to zero by definition). Most of the higher variability aromatic compounds occur above C_{11} alkane on this primarily non-polar stationary phase combination. It will be of interest to investigate these variabilities on other stationary phases polarities to gain some knowledge on the full relationship between structural molecular properties and temperature in a generalized context.

3.3. GC × GC inter-system changes

This portion of the project focused on comparisons between different GC × GC systems, as opposed to the changes that can occur within the same system – which were investigated in the previous section. The two key components that are likely to provide the most variability between GC × GC instruments are the type of modulator that is used (cryogenic or flow-based) and the type of stationary phase combinations that are assembled for the column set of the system. While we are aware that the other 2 building blocks of GC × GC systems (type of inlet unit, and type of detector) are also worthy to be included in a comparison study, our limitations in the types of inlets and detectors available determined the scope of our evaluations at this time.

3.3.1. GC × GC pairwise system comparisons

The RIC plot r^2 value was tested in a number of System comparisons once the analyses of the Century Mix had been performed in the participating laboratories. These pairwise comparisons (summarized in Table 7) were aimed at answering some questions with regards to specific GC × GC systems of particular interest, on the basis of the relative retention profiles of the Century Mix analytes over the bi-dimensional separation plane.

The first type of comparison involved the use of stationary phases combinations that are considered equivalent, such as the case of System 1 and System 2 in Table 7. They both use primary columns (Rxi-5 MS and BPX-5) that are classified as USP G27 phases, and both also use an

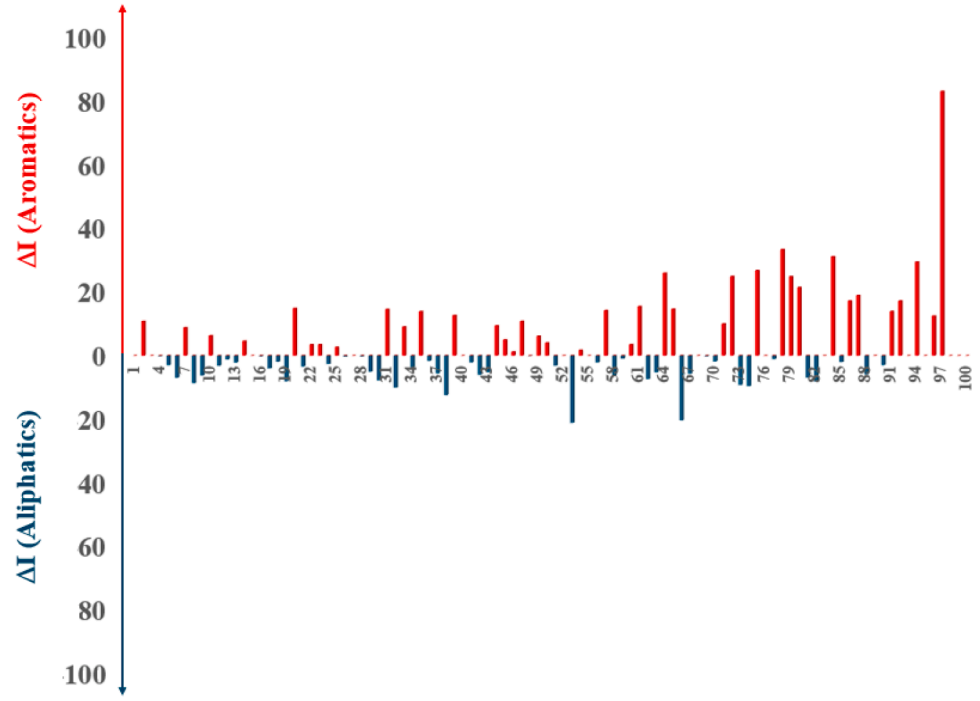


Fig. 5. Retention index variance plot on System 1 (non-polar/semi-polar column set) between temperature program rates of 2 °C/min and 20 °C/min. Compounds are listed in elution order, and separated between the aromatics (in red) and the aliphatics (in blue) in the Century Mix. All variances are reported in terms of the absolute values between the paired TPRI of each compound.

Table 7
Selected pairwise comparisons of GCxGC systems. See Table 2 for system details.

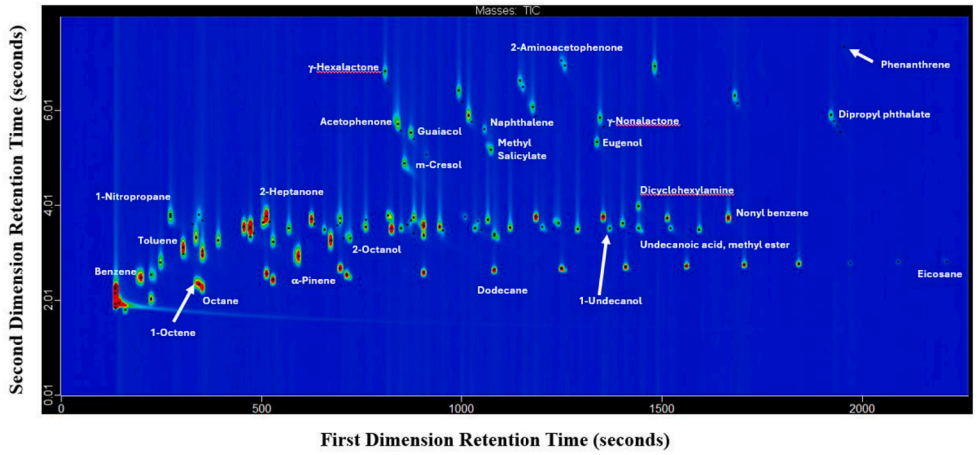
	GCxGC System	First Dimension	Second Dimension	Modulator	RIC plot r^2
1	System 1	Rxi-5 MS	Rxi-17	Quad Jet	0.9993
	System 2	BPX5	Rxi-17	Quad Jet	
2	System 1	Rxi-5 MS	Rxi-17	Quad Jet	0.9997
	System 3	Rxi-5 MS	Rxi-17	Peltier	
3	System 1	Rxi-5 MS	Rxi-17	Quad Jet	0.9997
	System 5	DB-5	Rxi-17 Sil MS	Flow (Paradigm)	
4	System 4	BPX5 MS	BPX50 MS	Flow (Insight FFF)	0.9996
	System 5	DB-5	Rxi-17 Sil MS	Flow (Paradigm)	
5	System 1	Rxi-5 MS	Rxi-17	Quad Jet	0.9989
	System 6	ZB-5 MS	VF-200	Quad Jet	
6	System 1	Rxi-5 MS	Rxi-17	Quad Jet	0.9325
	System 7	BPX50	Rxi-5	Quad Jet	

Rxi-17 column (USP G3) for the second dimension. They would be expected to produce very similar TPRI values, and the 0.9993 RIC plot r^2 value signifies that their operation can indeed be considered equivalent. Other types of pairwise comparisons that would fit in this category would involve the use of different phase film thicknesses, or the impact of how column bleed over the time of usage of a column set would affect the overall selectivity of the system.

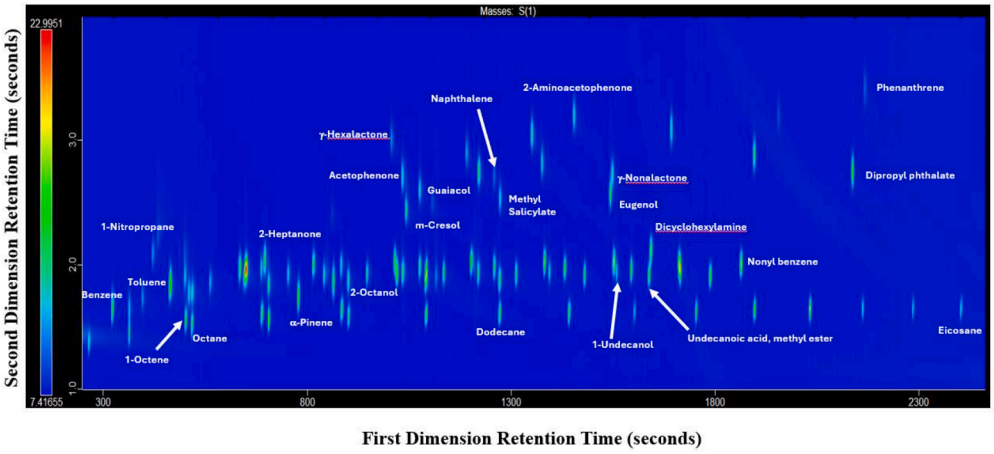
Another type of comparison involved the impact of modulator type on the separation. The comparison between two cryogenic modulators involves System 1 (Quad Jet modulator) and System 3 (Peltier modulation). The quad-jet modulator is operated inside the GC oven with cold jets that are liquid nitrogen cooled, whereas the Peltier modulator sits outside of the oven and does not use a consumable for the cooling process. The results, in terms of the relative positioning of the Century Mix peaks, produced an r^2 of 0.9997 which indicates a high level of consistency. A comparison was also done involving the two flow-based modulators that were used in this study: System 4 (Insight FFF, manufactured by SepSolve) and System 5 (Paradigm flow modulator, manufactured by LECO). The r^2 from this comparison was 0.9996, which again suggests a high level of correlation between the retention index values on the two platforms. The comparison between a cryogenic modulator (System 1) with a flow modulator (System 5) resulted in an r^2 of 0.9997. These results all provide a strong confidence that the type of GC × GC modulator does not generate significant deviations in the relative retention of target analytes. Fig. 6 shows some contour plot chromatograms for System 1 (Fig. 6A), System 5 (Fig. 6B) and System 4 (Fig. 6C) to provide a look into the consistency of the Century Mix profile on these three instruments. The relative positioning of the selected compounds is evident, in spite of the fact that they are collected at different modulation periods and that all the compounds have different retention time coordinates in all 3 chromatograms. Quantitative aspects of the separations are outside of the scope of this project, so no comments will be made about peak resolution or peak shape since no instructions were given to the team participants about inlet volumes, split ratios, or dilution factors. It is interesting to point out that the chromatogram for System 4 was phase-shifted by 0.66 s to visualize the peaks with no wraparound, as evidenced by the solvent peak near the top left of the contour plot. The relative peak positions of the compounds are not affected by this shift, however, due to the fact that the TPRI are calculated on the basis of the peak apexes in the raw chromatographic trace.

GCxGC systems that shared an equivalent first dimension column but had slightly different secondary columns were also compared. In this category, System 1 was compared to System 6. The non-polar first dimension columns are both classified as USP G36 stationary phases, but the semi-polar secondary columns have different USP classification: Rxi-17 is a USP G3 phase, whereas the trifluoropropyl VF-200 is a USP G7 phase. The RIC plot r^2 value of 0.9989 suggests that, while relatively close in character, these two systems cannot be considered equivalent because there are a significant number of ΔI values that exceed the threshold for strong match consideration.

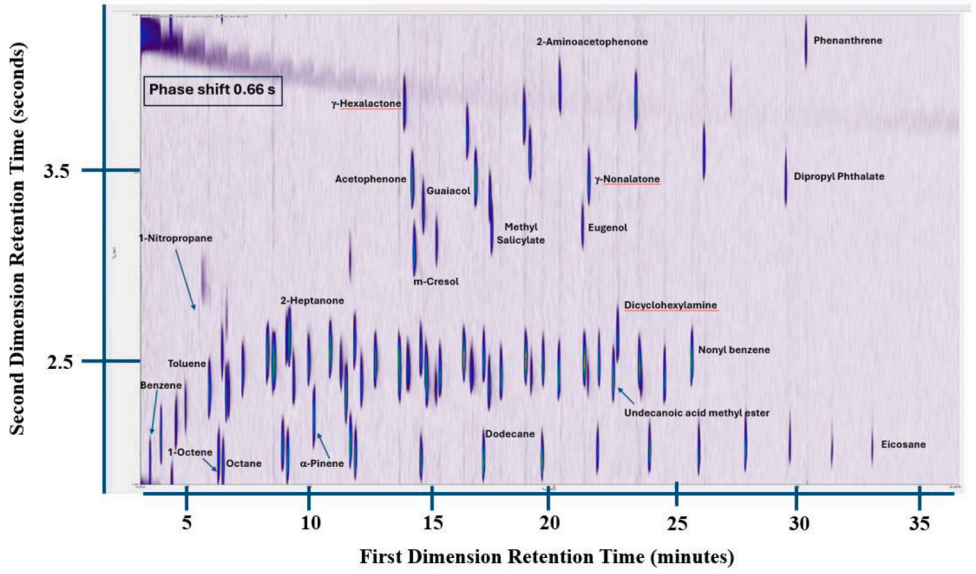
One final case involved the comparison of a non-polar/semi-polar column set (System 1) to its reversed combination (semi-polar/non-polar, System 7). The lower RIC plot r^2 value of 0.9325 signifies that



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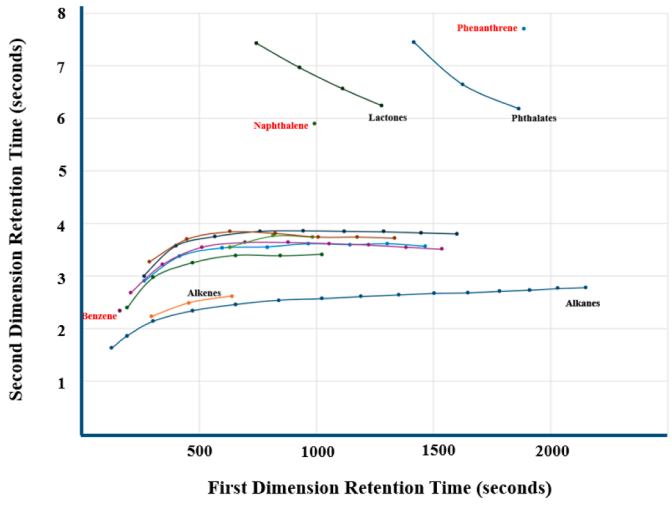


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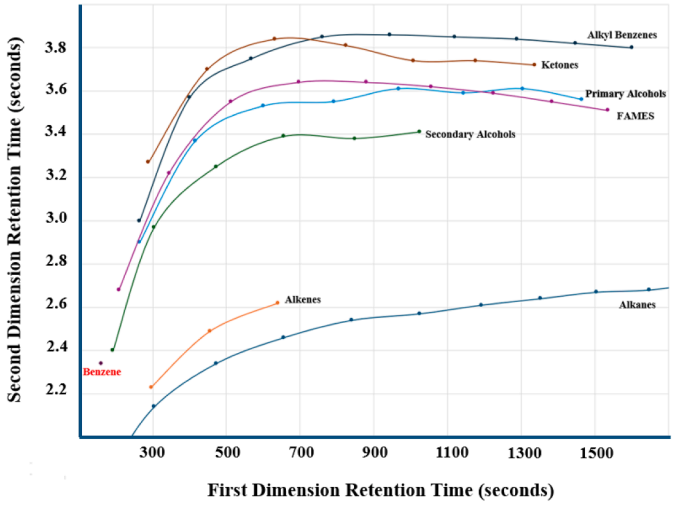


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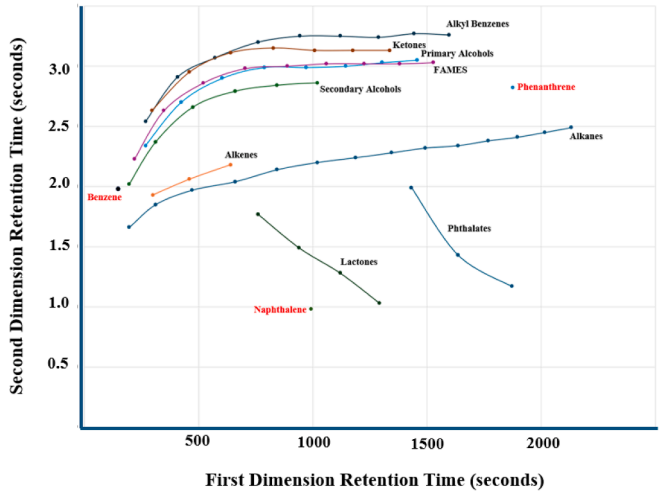
Fig. 6. Century Mix contour plot profiles on 3 GC × GC systems. All 3 systems are using non polar/semi-polar column sets (see Table 2 for details). 6A. System 1. Quad-jet cryogenic modulator. Modulation period: 8-seconds. TOF MS detector. 6B System 5. Paradigm flow modulator. Modulation period: 4-seconds. FID detector. 6C. System 4. FFF flow modulator. Modulation period: 4.5-seconds. FID detector.



A



B



C

Fig. 7. Century mix peak apex plots of selected homologous series of compounds on 2 different. GC × GC systems. 7A. System 1 (non-polar/semi-polar column set, see Table 2 for details), operated with quad-jet modulation (modulation period: 8 s). 7B Closeup region (between 2.0 and 4.0 s in the second dimension) of the peak apex plot shown in Fig. 7A. 7C. System 3 (non-polar/semi-polar column set, see Table 2 for details), operated with a Peltier modulator (modulation period: 4 s).

Table 8
GC × GC second dimension elution order system classification. Normal orthogonality systems are the ones where alkanes elute before the alkenes. Reversed orthogonality systems are the one where the alkenes elute before the alkanes. Hybrid systems are the ones where the alkanes don't elute first or last in the elution order.

	Systems 1–5	System 6	Systems 7–9	System 10	System 11
^{1}D	Non-polar	Non-polar	Semi-polar	Semi-polar (I)	Semi-polar (II)
^{2}D	Semi-polar (I)	Semi-polar (II)	Non-polar	Semi-polar (II)	Semi-polar (I)
	Alkanes	Alkanes	Phthalates	Lactones	Alkyl benzenes
	Alkenes	Alkenes	Lactones	Ketones	Alkenes
	2° Alcohols	Alkyl benzenes	Ketones	FAMES	1° Alcohols
	1° Alcohols	2° Alcohols	FAMES	2° Alcohols	Alkanes
	FAMES	1° Alcohols	1° Alcohols	Phthalates	Phthalates
	Ketones	FAMES	Alkyl benzenes	Alkanes	2° Alcohols
	Alkyl benzenes	Ketones	2° Alcohols	Alkenes	FAMES
	Lactones	Phthalates	Alkenes	1° Alcohols	Ketones
	Phthalates	Lactones	Alkanes	Alkyl benzenes	Lactones

these column sets are very different in selectivity, and that RI information obtained on one of these systems cannot be used to predict or locate compounds on the other. As more different GC × GC systems are investigated with this approach, a better picture of selectivity will become more evident. It should also be said that other synthetic mixtures could be used with this approach, so long as the target analytes are run on both systems so that the comparisons are completed on the basis of the same information for all compounds of interest.

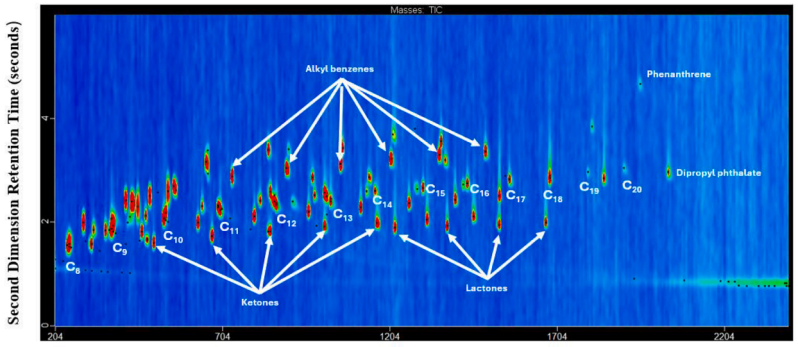
3.3.2. GC × GC column set classifications by secondary column elution order

Even though the first dimension carries the bulk of the retention index information in GC × GC, it is the ordering provided by the secondary dimension that provides the direction of the polarity gradient that is important for column classification and compound identification in non-targeted analyses. Fig. 7 shows a series of peak apex plots of the Century Mix on a variety of stationary phase combinations. The order in which each series elutes can be considered a signature of the stationary phase combination. Fig. 7A is a peak apex plot of a system (System 1 from Table 2) with a non-polar (Rxi-5 MS) first dimension column coupled to a semi-polar (Rxi-17 MS) secondary column. The first two eluting series are the alkanes followed by the alkenes, and the last two eluting series are the lactones and the phthalates. The other homologous series elute in a narrow secondary elution time (2 s), and Fig. 7B shows a closeup of this region of the chromatogram. One can see that the fatty acid methyl esters (FAMES) and the primary alcohols switch elution order during the course of the run, and a similar order swap occurs between the alkylbenzenes and the ketones. Fig. 7C is a peak apex chromatogram of a different non-polar/semi-polar system (System 3 from Table 2) that used a BPX-5 primary column and an Rxi-17 column. In System 3 the modulation time was 4 s (compared to the 8-second modulation time used in System 1), which explains why the lactones and the phthalates appear at the bottom of the chromatogram because they are wrapped-around. The elution pattern of the other homologous series is the same as those shown in Fig. 7A, including the series that flip during the course of the run. These patterns were similar for all 5 non-polar/semi-polar stationary phase systems investigated in this study, which is summarized in Table 8. It is particularly satisfying to observe that the elution patterns of the flow systems (Systems 4 and 5) are essentially the same as their cryogenic counterparts (Systems 1,2, and 3) because this validates the use of the Century Mix for the "qualitative" assessment of the selectivity character of any stationary phase combination class.

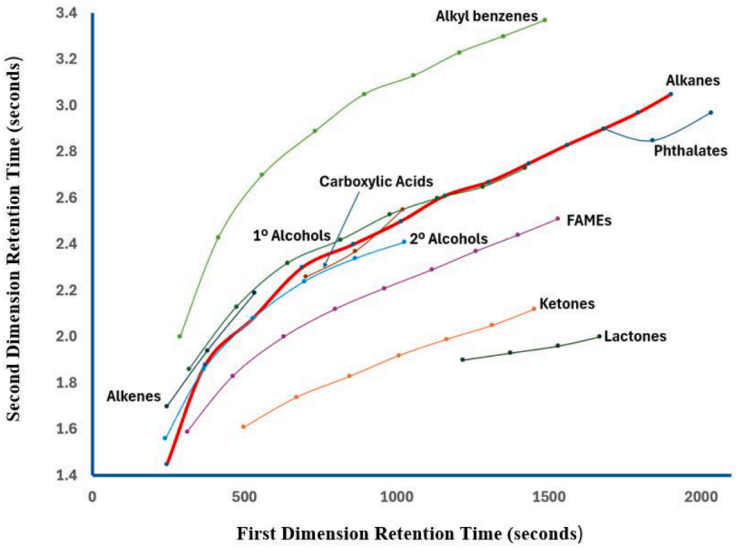
The elution orders of the 3 semi-polar/non-polar systems (Systems 7, 8, and 9 in Table 2) are also shown in Table 8, and they provide additional useful information for the utility of the Century Mix in characterizing GC × GC stationary phase combinations. In these systems, the elution order is indeed "reversed" from the patterns seen in the "normal orthogonality" systems, with the phthalates and lactones eluting, early, and the alkenes and alkanes eluting late in the secondary dimension. The fact that there are small differences between the elution profiles of systems in the same general class is attributable to the polarity differences that exist between stationary phases that are categorized in the same general polarity class (e.g., semi-polar stationary phases like Rtx-17 and VF-200). For instance, in System 6 the alkylbenzenes elute in third position (behind the alkanes and alkenes) whereas in all the other normal orthogonality systems they elute in 7th position. It is also noticeable that the phthalates elute ahead of the lactones in System 6, whereas in all the other normal orthogonality systems (Systems 1–5) the lactones elute ahead of the phthalates.

The evaluation of new (or previously non-reported) stationary phase combinations is also possible through the use of the Century Mix elution order method, and the results can be seen in Table 8 for Systems 10 and 11(chromatographic information is also provided in Fig. 8). In both these systems, two semi-polar stationary phases were coupled: VF-200/Rtx-17 for System 10, and Rtx-17/VF-200 in System 11. The secondary dimension gradient in both these systems is significantly different from the others because the alkanes are neither the first series to elute (as they do in all the normal orthogonality systems) nor the last series to elute (as they do in all the reversed orthogonality systems). The only other published demonstration of this type of column set system was done by Seeley et al. using a DB-210/HP-50 column ensemble (using a flow modulator), where they showed FAME compounds eluting below the hydrocarbons in a diesel sample [38]. The elution order shown for System 10 (using a cryogenic modulator) is consistent with the results of the Seeley paper because they are both the results of GC × GC systems using the trifluoropropyl semi-polar stationary phase as the primary column, and showing the elution of FAME compounds below the saturated hydrocarbons (as shown in Fig. 8B). The added value of the Century Mix separation is the fact that a better understanding of the secondary column selectivity gradient is gained through the use of the same set of homologous series that are used to characterize other column combination systems. It is now clear that FAMES are not the only series that elutes below the alkanes, but in fact one of 5 functional groups to do so. Moreover, the coupling of the reversed combination (System 11) results in an elution order in which the FAMES now elute above the alkanes (as shown in Fig. 8C), just as the other four homologous series. System 10 is classified as a "normal hybrid" column set to reflect the fact that the alkanes have other homologous series eluting above and below them, and to indicate that the direction of the separation gradient is similar to what is observed when the primary column is less polar than the secondary column due to the fact that the alkenes elute after the alkanes. Similarly, System 11 is classified as a "reversed hybrid" column set because the alkenes elute before the alkanes.

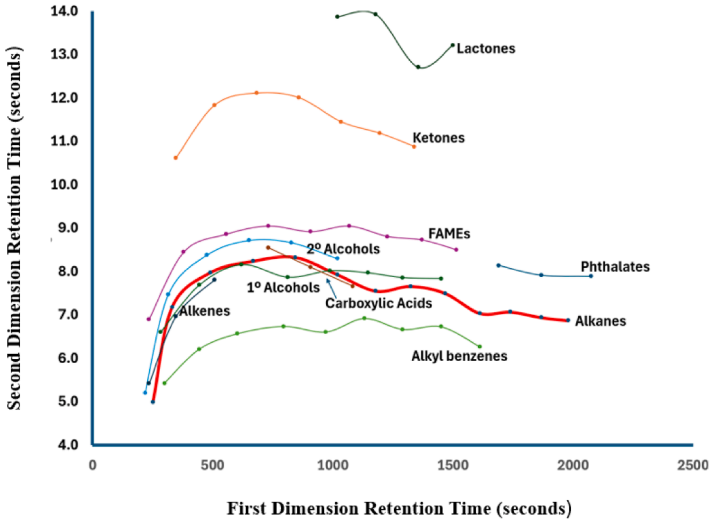
One final note with regards to new column combinations: it is important to establish the elution order at modulation periods where there is no wrap-around so that the true elution order can be observed (once the elution order is known, other modulation periods can be used in case better resolution is needed). A practical way to determine if there is wrap-around in the chromatogram is to have two runs at two different modulation periods. Compounds that are not wrapped-around will have the same secondary retention time, whereas compounds that are wrapped-around will show a noticeable shift in retention time. It is also important to choose modulation periods that are not multiples of each other, in order to avoid aliasing synchronicity, which means that a peak may appear at the same retention time because the modulation period is in sync with the secondary retention time.



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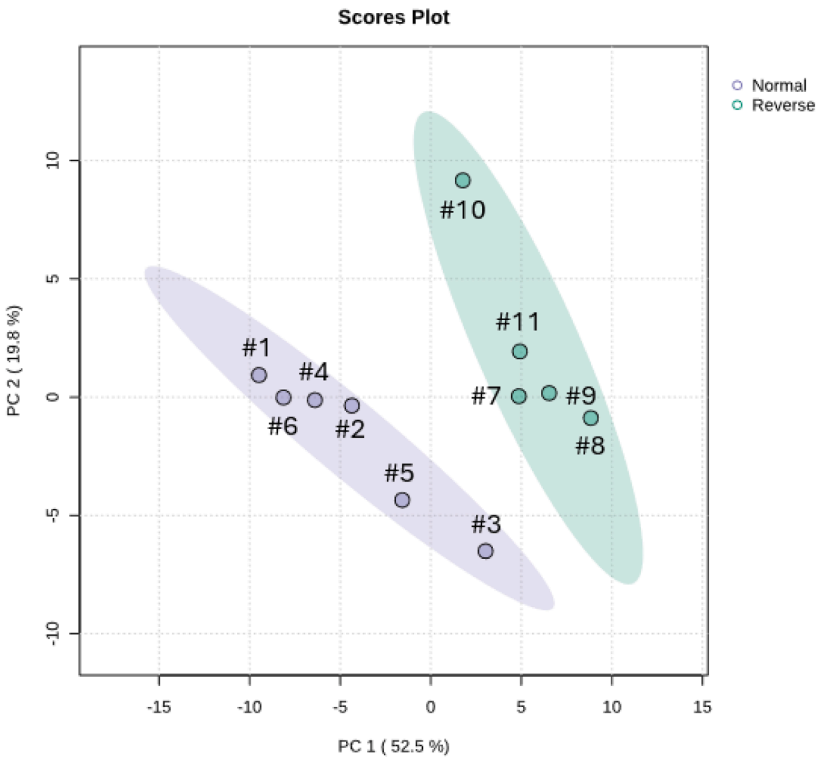


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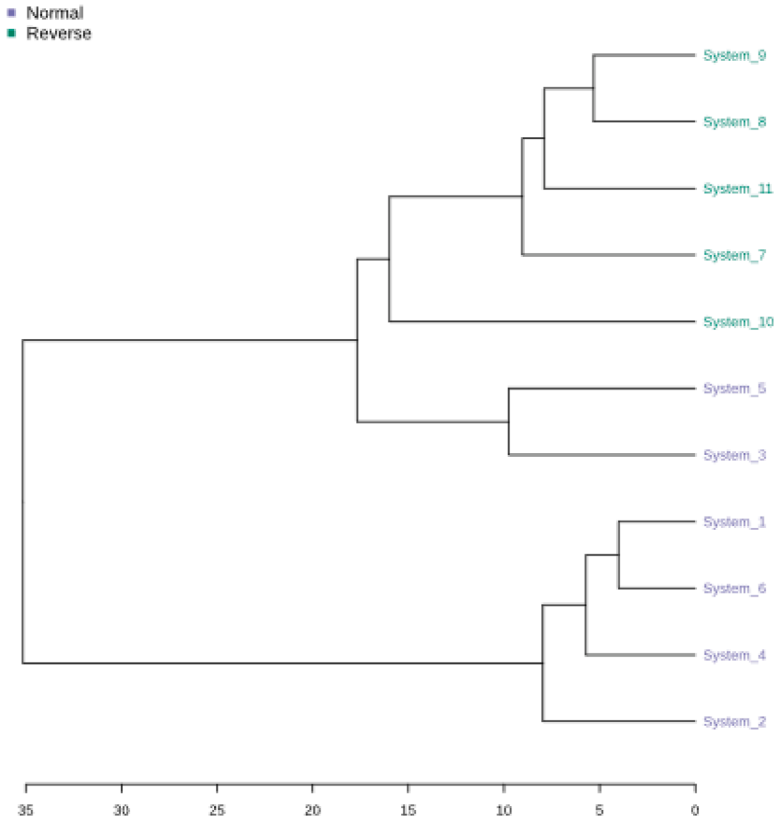


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Fig. 8. Hybrid orthogonality GC × GC column set Century Mix profiles. 8A. Contour plot chromatogram of normal hybrid orthogonality system (System 10), with lactone and ketones eluting below the alkanes, and alkyl-benzenes eluting above the alkanes. 8B Peak apex plot of contour plot chromatogram shown in 8A. 8C. Peak apex plot of a normal reversed hybrid orthogonality system (System 11), with lactone and ketones eluting above the alkanes, and alkyl-benzenes eluting below the alkanes.

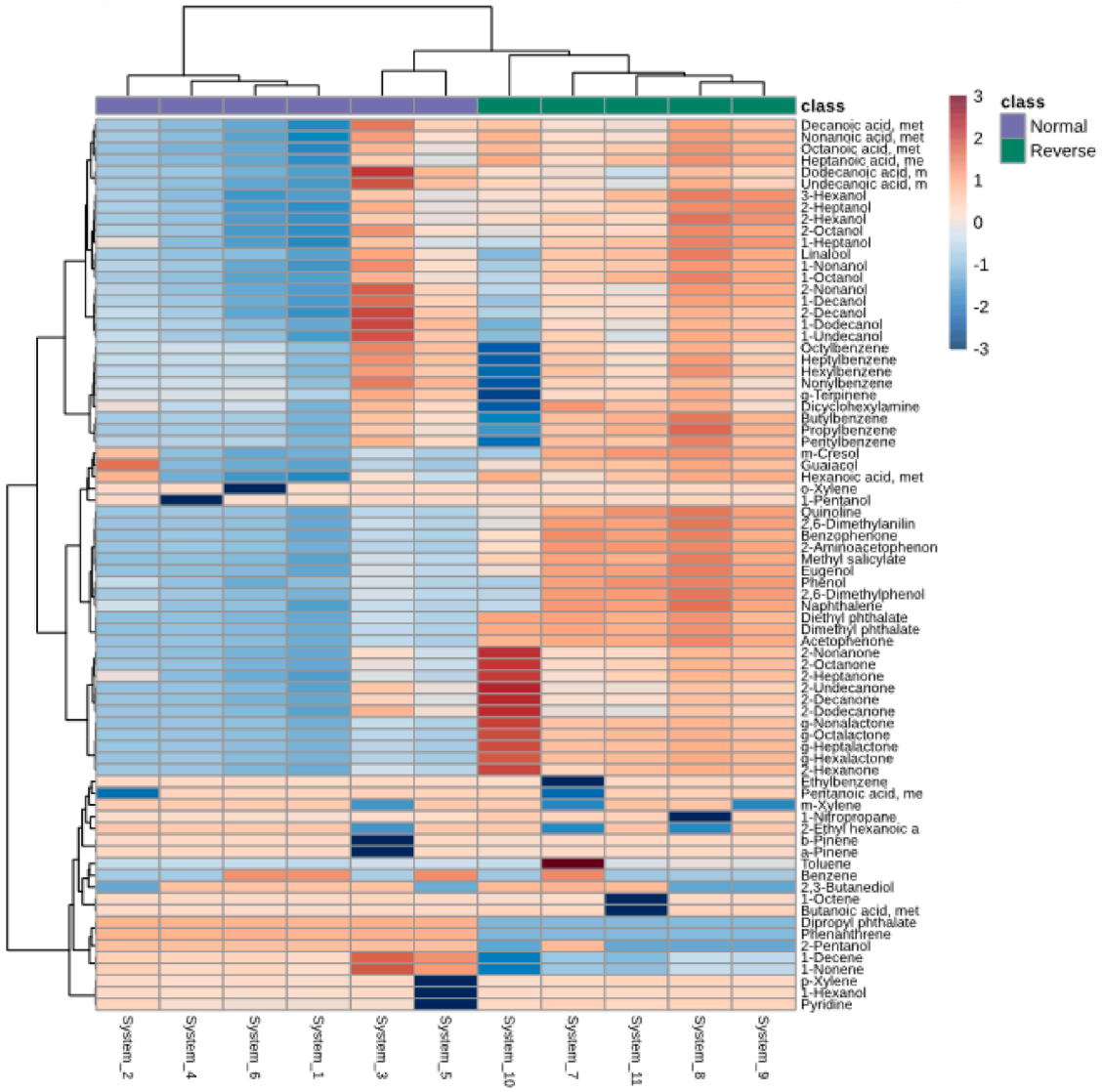


A



B

Fig. 9. Classification of GC × GC Systems (see Table 2 for system details). 9A. PCA plot. 9B Dendogram plot. 9C. Heatmap plot. Color legend indicates the intensity of the variance (above or below the mean).



C

Fig. 9. (*continued*).

3.3.3. GC × GC system comparisons using PCA and Heatmap plots

Although pairwise comparisons are useful in specific instances as described in Section 3.3.1, it is also important to be able to compare sets of profiles as a group. A matrix was generated with all the TPRI values from all the systems involved in this study for each Century Mix analyte. This data was then normalized and z-score autoscaled to prepare it for PCA processing.

The results of these inter-system calculations are depicted in Fig. 9. Fig. 9A presents a PCA plot that illustrates the separation between the two main types of GC × GC systems. The PCA plot effectively captures the variation in system profiles, clustering the systems based on their underlying retention characteristics. This dimensionality reduction technique highlights distinct groupings, with the hybrid systems (Systems 10 and 11) showing a closer association with the reversed column set systems. This observation emphasizes the dominant influence of the primary column's stationary phase on the overall separation character in GCxGC, despite the secondary column playing a crucial role in chromatogram structuring.

Complementing the PCA plot, the dendrogram plot in Fig. 9B provides a hierarchical representation of system similarities. By grouping systems based on their retention profiles, the dendrogram adds another layer of understanding to the inter-system relationships. The close clustering of hybrid and reversed column systems in the dendrogram further corroborates the PCA findings. It is also important to point out that there are two "normal" column pairs (Systems 3 and 5) that seem to be clustering with the "reverse" column sets, and we do not have a good explanation for this result at the moment. The study of more stationary phase combinations in future studies will be critical is establishing a wider scope of the selectivity similarities and differences that are outlined in Figs. 9A and 9B

Finally, Fig. 9C presents a heatmap plot that provides compelling insights into the relationships among the various compounds examined in this study across the series of column sets. The heatmap visually encodes relative retention differences, offering a clear and intuitive comparison of homologous compounds within a given set. This level of detail facilitates the identification of patterns or trends in compound behavior, whether through variations in structural properties or through interactions with specific column chemistries. Moreover, the ability to

compare individual target analytes across multiple column systems enhances the analytical depth of the study. This comparison becomes particularly valuable as more column configurations are evaluated, enabling researchers to systematically assess their performance and suitability for specific applications. Additionally, the data's normalization to standardized conditions ensures consistency allowing for longitudinal comparisons over time in an efficient manner. This feature makes the heat map a useful tool for immediate analysis and also for ongoing method development and optimization.

4. Conclusion

The use of the raw GC × GC trace method for the calculation of retention index data has yielded some solid results that can be extended to compounds beyond the Century Mix, and the use of the RIC r^2 metric has been demonstrated to assess the similarity between profiles of relative retention information generated on different systems. We plan to further associate the use of the RIC plot r^2 with the retention index variance histograms to provide a more complete assessment of system similarity from both the full target compound list as well as the individual compound perspective. We anticipate that a database of retention index information can be developed that will be useful for non-targeted analysis.

Within the operation of a single GC × GC system, it was found that variations in flow rate data acquisition rate and modulation period do not significantly impact the relative retention profiles of the chromatograms. Not surprisingly, the biggest operating factor in the variation of the TPRI values is the temperature programming rate, although rate fluctuations below 10 °C/min result in retention index variations that are below the 12 index unit threshold. We plan on performing further investigations to explore the relationship between open-chain and closed-chain organic compounds on retention index variability in a larger set of GC × GC systems.

In terms of the comparisons between different GC × GC systems, the method found excellent correlation (RIC plot r^2 above 0.999) between cryogenic systems and flow-based modulation systems. The distinctions between column sets are also clearly established through the ΔTPRI information, both in pairwise comparisons as well as comparisons involving larger groups of data (as reflected in the PCA and heatmap plots for the cohort of systems that were part of this study). A better understanding of the "hybrid" column sets is needed because they are currently associated with the reversed column set systems even though their elution orders are different than the elution orders of the other systems in that class. We intend to investigate more column sets in our future work to better define the selectivity differences between these systems.

Supplemental Tables are provided in the adjoining Excel File.

Table S1. Detailed operating conditions for all 11 systems used in this study.

CRediT authorship contribution statement

Jean-Marie D. Dimandja: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Conceptualization. **Jeffrey C. Archer:** Writing – review & editing, Methodology, Funding acquisition. **Erica L. Bakota:** Writing – review & editing. **Jeffrey D. Bruce:** Writing – review & editing, Methodology. **John A. Casanova:** Writing – review & editing, Methodology, Investigation. **Eric A. Posenecker:** Writing – review & editing, Investigation. **Anaïs Rodrigues:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Data curation. **Djulia Bensaada:** Writing – review & editing, Investigation, Data curation. **Pierre-Hugues Stefanuto:** Writing – review & editing, Visualization, Supervision, Project administration, Methodology, Conceptualization. **Jean-François Focant:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Daniel R. Struk:** Writing – review & editing, Methodology, Data curation. **Rizky Ilhamsyah:** Writing – review & editing, Methodology, Data curation. **Peter J. Hesketh:** Writing – review & editing, Supervision, Project administration. **Christina N. Kelly:** Writing – review & editing, Investigation, Data curation. **Joseph E. Binkley:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Giorgia Purcaro:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Aleksandra Gorska:** Writing – review & editing, Methodology, Investigation. **Damien Eggermont:** Writing – review & editing, Methodology, Investigation. **Marie Vaccaro:** Writing – review & editing, Methodology, Investigation. **Pascal Cardinael:** Writing – review & editing, Project administration, Funding acquisition. **Christopher A. Heist:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The Author G. Purcaro. given her role as Associate Editor in J. Chromatography Open, had no involvement in the peer review of this article and had no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to another journal editor.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcoa.2026.100345.

Data availability

Data will be made available on request.

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