Supplementary Information for

An Intelligent Multidimensional Purity Analysis and Confirmation Tool for Multiple Attribute Analysis

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Contents

1. Materials and Methods

Chemicals.

Reagent grade dimethyl sulfoxide (99.7 %), (*S*)-(-)-5-(2-pyrrolidinyl)-1H-tetrazole (96 %), 4-nitrobenzaldehyde (98 %), acetonitrile (99.8 %), and diethyl amine (99.5 %) were purchased from Sigma-Aldrich Chemical Company and used without further purification. Acetone (99.8%) was purchased from Fisher Scientific and used without further purification.

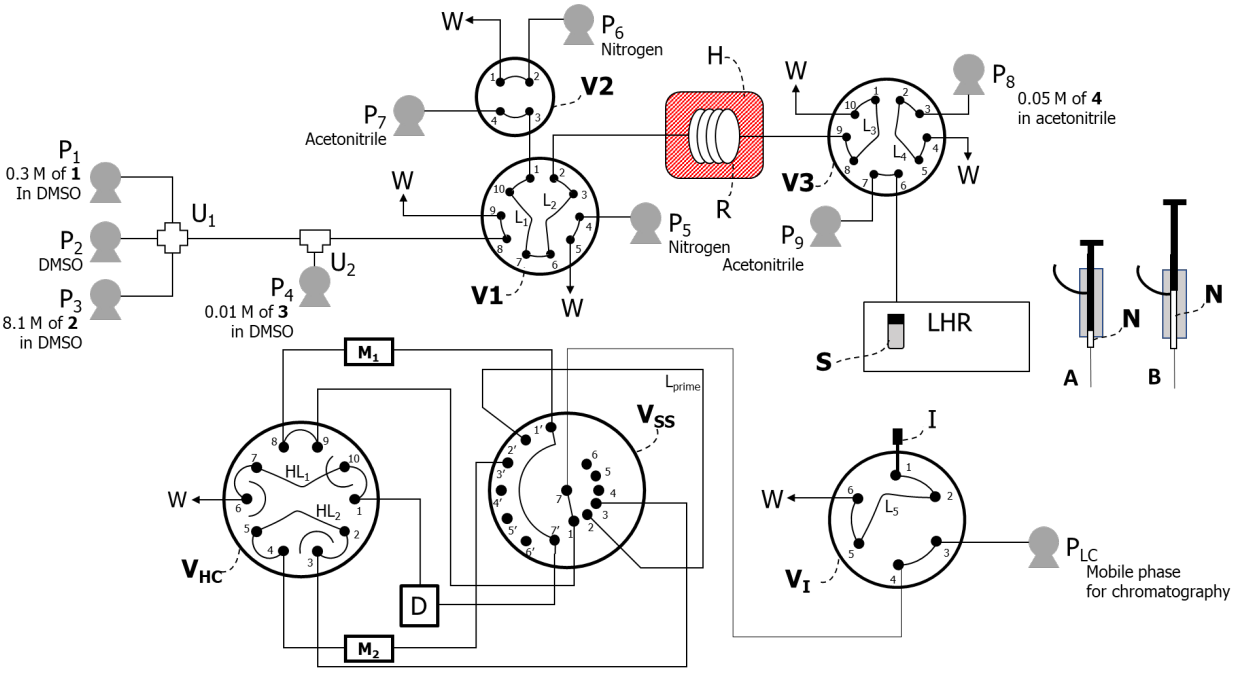
HPLC-grade hexanes, 2-propanol, water, and ethanol were purchased from Sigma-Aldrich Chemical Company.

Methanol solution of racemic oxazepam (1 mg/mL) was purchased from Sigma-Aldrich Chemical Company and used without further purification.

2. Supplementary Text

Reaction, Sampling, and Analysis.

Figures S1 and S2 illustrate various modules of the entire setup at various configurations for reaction, sampling, and analysis.



Legend. P_1, P_2, P_3, P_4 : reagent pumps; P_5, P_6 : pumps for forming nitrogen gas boundaries for reaction plugs; P_7 : pump for transporting reaction plugs; P_8 : pump for adding internal standard solution; P_9 : pump for transporting analyte to liquid handling robot; P_{LC} : LC pump; V_1 and V_2 : valves for constructing reaction plugs; V_3 : auto-sampling valve; V_I : LC injector valve; V_{SS} : column-selector valve; V_{HC} : heart-cut valve; LHR : liquid handling robot; N : injector needle; I : Injection port; S: sampling vial; R : plug-flow reactor; H : heater; L_1 and L_2 : loops for constructing reaction plugs; L_3 : sampling loop; L_4 : loop for adding internal standard; L_5 : injection loop; L_{prime} : priming loop; HL_1 and HL_2 : heart-cut loops; U_1 and U_2 : unions for combining reagent streams from P_1-P_4; W : Waste. M_1 and M_2 : LC columns; D : detector.

Figure S1. Reaction, sampling, and analysis setups wherein valves V_1, V_2, and V_3, which used for reaction and sampling, are in the first configuration; valve V_I, which is used for injection into analytics, is in the load configuration. Valves V_{SS} and V_{HC}, which are used in the multidimensional analysis setup, are shown to move fluid through M_1 column. Sample delivery and injection modes of the injector needle are also shown.

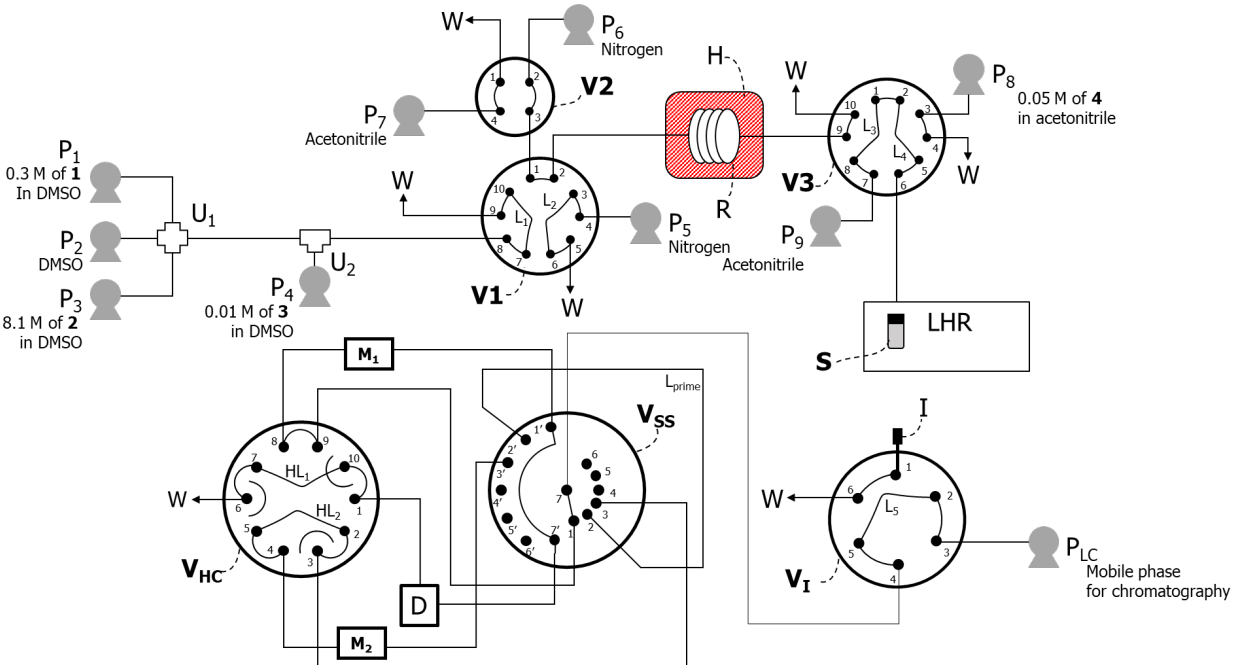


Figure S2. Reaction, sampling, and analysis setups wherein valves V_1, V_2, and V_3, which used for reaction and sampling, are in the second configuration; valve V_I, which is used for injection into analytics, is in the inject configuration. Valves V_{SS} and V_{HC}, which are used in the multidimensional analysis setup, are shown to move fluid through M_1 column.

Reaction Setup.

Three programmable syringe pumps (P_1, P_2, and P_3; New Era) were connected to a 4-way union (U_1; SciPro). The fourth line of U_1 was connected to a 3-way union (U_2; SciPro), which was also in fluid communication with a fourth programmable syringe pump (P_4; New Era). The third line of U_2 was connected to a ten-port, two-position valve (specifically, port 10 of valve V_1; VICI). Two loops (L_1 and L_2; SS, 0.03" ID, 50 μL each, respectively; VICI) were mounted between ports 3 and 6, and between ports 7 and 10 of V_1. Port 1 of V_1 was connected to a four-port, two-position valve (specifically, port 3 of valve V_2; VICI). Two additional programmable syringe pumps (P_5 and P_6; New Era) were connected to port 4 of V_1 and port 2 of V_2, respectively. Port 4 of V_2 was connected to a programmable syringe pump (P_7; New Era). Port 1 of V_2 and ports 5 and 9 of V_1 were connected to waste. All flow-paths among valves (V_1 and V_2), pumps (P_1 to P_7) and waste were made of PFA tubes (0.03" ID; SciPro).

Port 2 of V_1 was connected to individual reactor coils (R; SS, 0.01" for a 50 μL reactor; 0.02" for a 65 μL reactor, and 0.03" for a 250 and a 1000 μL reactors; VICI), which were immersed in a bath filled with metal beads (Heidolph) that can conduct heat from a hot-plate stirrer (H; IKA) to reactor R. The other end of R was connected to a ten-port, two-position valve (specifically, port 9 of valve V_3; VICI). Port 10 of V_3 was connected to waste.

Sampling Setup.

Valve V_3 served as the bridge between the reaction setup and the sampling setup. Ports 1 and 8 and ports 2 and 5 of V_3 were connected to two additional loops (L_3 and L_4; SS; 0.03" ID, 20 μL each; VICI), respectively. Two programmable syringe pumps (P_8 and P_9; New Era and CTC, respectively) were connected to ports 3 and 7 of V_3, respectively. Port 6 of V_3 was connected to a flexible PFA tube (0.03" ID, 1000 μL; CTC) that was connected to a configurable injector needle (N; Figure S1). Needle N has a side port, which could be closed by a liquid handling robot (LHR; CTC) in one mode (mode A, which was used for injection; Figure S1) and opened in another mode (mode B, which was used for sample delivery; Figure S1). A robo-tic arm of LHR was programed to move on top of a capped sampling vial (S), pierce through the cap of vial using N, and deliver a reaction specimen into S using mode B of needle N. LHR was also equipped with a needle cleaning station (not shown). Port 4 of V_3 was connected to waste.

Analysis Setup (4-hydroxy-4-(4-nitrophenyl)-butan-2-one).

Injector needle N (Figure S1) was also used for analytical injections. During analytical injections, the side-port of N was closed (mode A; Figure S1). Needle injected analytical samples into a vertical port (specifically, port 1 through vertical cup I) of a six-port, two-position valve (valve V_I; CTC). An injection loop (L_5; SS, 0.01" ID; 20 μL; VICI) was connected between ports 2 and 5 of V_I. Port 2 of V_I was connected to waste. Port 3 of V_I was connected to a quaternary LC pumps of Agilent 1260 Infinity LC system with built-in degasser. Port 4 of V_I was connected to a fourteen-port, six-position stream selector valve (specifically, port 7 of valve V_{SS}; Agilent). Ports 2 and 2' were connected by a loop (L_{prime}; VIPER, 0.005" ID; Thermo-Fisher Scientific). Port 7' of V_{SS} was connected to the upstream end of PDA detector D (Agilent), which is a part of the 1260 system. Ports 1' and 3' were connected to a Luna 5 μm Silica LC column (M_1; 100 x 4.6 mm; Phenomenex) and a Lux 5 μm Cellulose-2 LC column (M_2; 250 x 4.6 mm; Phenomenex), respectively. Downstream ends of columns M_1 and M_2 were connected to a twenty-position, ten-port valve (specifically, ports 8 and 4 of valve V_{HC}, respectively; VICI), which was equipped with two heart-cut loops (HL_1 and HL_2; SS; 0.03" ID; 100 μL and 140 μL, respectively; VICI). Port 1 of V_{HC} was connected to the downstream end of the PDA detector. Ports 3 and 9 of V_{HC} were connected to ports 3 and 1 of V_{SS}, respectively. Port 6 of V_{HC} was connected to waste. Ports 4, 4', 5, 5', 6, and 6' were closed by dead-end nuts. All analytical valves (V_I, V_{SS}, and V_{HC}), pump P_{LC}, and detector D were connected using VIPER piping (0.005" ID; Thermo-Fisher Scientific). Valves (V_1, V_2, V_3, and V_I, and V_{SS}) were equipped with standard rotor that simultaneously connect adjacent ports of the stator. Valve V_{HC} was equipped with a custom rotor with 8 curved slots that connect a specific set of ports on its stator leaving certain ports disengaged from pump P_{LC}.

All twenty positions (C-1 to C-20), which are 18° apart from each other, of valve V_{HC} are shown in Figure S3. Owing to the unique design of eight curved slots on the rotor (US provisional application 63/045,388) and the unique arrangement of V_I, V_{SS}, V_{HC}, and D (US granted 10, 585, 071), the multidimensional chromatographic platform is capable of delivering six unique configurations that are capable of arresting any portion of the eluent from a chromatographic run and releasing the portion after waiting any amount of time without ever stopping the ongoing chromatographic run. Five functional configurations of valve V_{HC} are:

The first configuration, wherein the eluent carrying an analytical sample moves from M_1 to HL_1 without establishing fluid communication between P_{LC} and HL_2 (shown in Figure S1 or S2).

The second configuration, wherein the eluent carrying an analytical sample moves from M_1 to HL_2 without establishing fluid communication between P_{LC} and HL_1 (shown in Figure S1 or S2).

The third configuration, wherein the eluent carrying an analytical sample moves from M_1 to HL_2 while maintaining fluid communication between P_{LC} and HL_1 (shown in Figure S1 or S2).

The fourth configuration, wherein the eluent carrying an analytical sample moves from M_2 to HL_1 without establishing fluid communication between P_{LC} and HL_2 (shown in Figure S1 or S2).

The fifth configuration, wherein the eluent carrying an analytical sample moves from M_2 to HL_2 without establishing fluid communication between P_{LC} and HL_1 (shown in Figure S1 or S2).

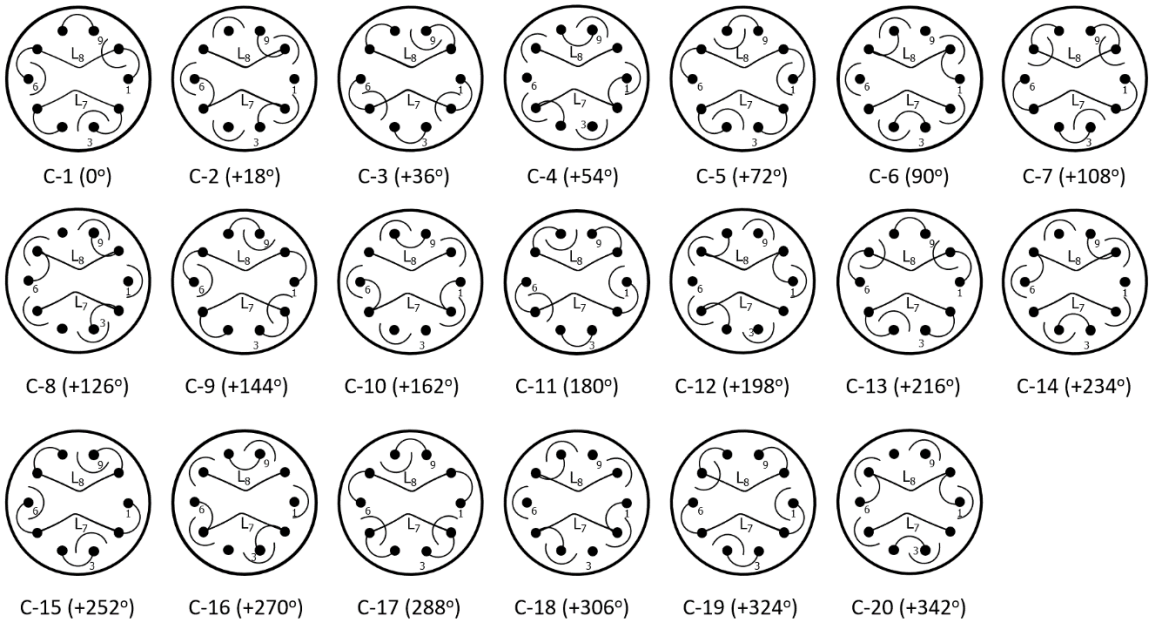
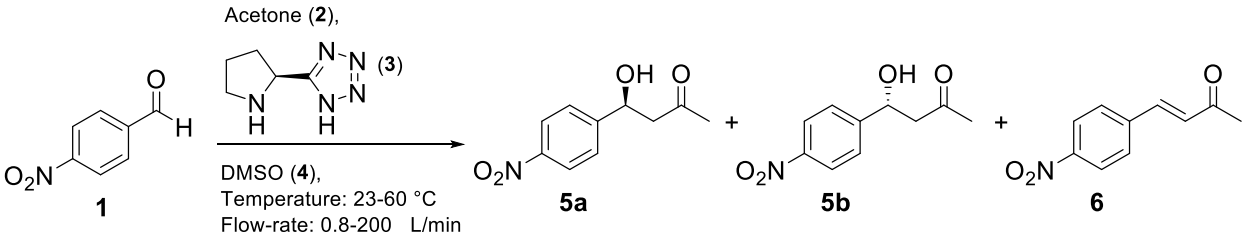


Figure S3. Twenty positions of valve V_{HC} were shown. The rotor was moved in counter-clockwise direction by 18° to achieve these positions.

Reaction Scheme (4-hydroxy-4-(4-nitrophenyl)-butan-2-one).

The aldol reaction shown in Scheme S1 was used as a model proof-of-concept reaction.



Scheme S1. Asymmetric synthesis of 4-hydroxy-4-(4-nitrophenyl)-butan-2-one.

Operating Procedure.

All flow-paths were primed with acetonitrile prior to operating the entire setup. Minute variation in elution time is intelligently adjusted by the operating software (screenshot of GUI is shown in Figure S4).

A 0.3 M solution of 4-nitrobenzaldehyde in DMSO, a 8.1 M solution of acetone in DMSO, and a 0.1 M solution of (*S*)-(-)-5-(2-pyrrolidinyl)-1H-tetrazole in DMSO were aspirated in three Norm-ject luer-lock plastic syringes (3 mL; Fisher Scientific), which were mounted on pumps P_1, P_3, and P_4. A Norm-ject syringe (5 mL; Fisher Scientific), which was loaded with DMSO, was mounted on pump P_2. Valves V_1, V_2, and V_3 were configured to remain in first positions (as shown in Figure S1). In this position (the first position of V_1 and V_3), V_1 and V_3 connect ports 1 and 10, 2 and 3, 4 and 5, 6 and 7, and 8 and 9 of respective valves. Valve V_2 connects ports 1 and 2, and 3 and 4 in this position (the first position of V_2). All reagents and DMSO were first flowed through U_2 to W via ports 8 and 9 of V_2 at a net flow-rate of 60 μL/min for 1.5 min. Individual flow-rates of pumps vary based on a specific stoichiometry of a reaction condition under trial (Table S1).

Table S1. Flow-rates of reagent pumps (P_1-P_4) to conduct all sixteen reaction optimization experiments.

	Flowrate (*m*L/min)			
Entry	P_1	P_2	P_3	P_4
1	10	10	10	30
2	10	5	15	30
3	10	15	5	30
4	10	25	10	15
5	10	10	10	30
6	10	5	15	30
7	10	0	10	40
8	10	10	10	30
9	10	10	10	30
10	10	10	10	30
11	10	10	10	30
12	10	10	10	30
13	10	10	10	30

14	10	10	10	30
15	10	10	10	30
16	10	10	10	30

Table S2. Tabulated process parameters and quality attributes of the reaction of Scheme S1.

Entry	Equiv. of **2**	Equiv. of **3**	Reaction temp. (°C)	Reactor ID (cm)	Reactor length (cm)	Flow-rate (μL/min)	Residence time (min)	Yield of **6** (%)	Yield of **5** (%)	Enantiomeric excess (%)
1	27	0.10	60	0.076	219.3	200	5	2	17	70
2	40.5	0.10	60	0.076	219.3	100	10	4	52	72
3	13.5	0.10	60	0.076	219.3	100	10	3	25	79
4	27	0.50	60	0.076	219.3	100	10	3	37	76
5	27	0.10	60	0.076	54.8	25	10	5	52	76
6	40.5	0.10	60	0.076	54.8	25	10	3	42	74
7	27	0.13	60	0.076	54.8	25	10	4	41	75
8	27	0.10	60	0.076	219.3	100	10	5	56	73
9	27	0.10	60	0.076	219.3	50	20	2	29	72
10	27	0.10	60	0.076	219.3	33.3	30	7	87	73
11	27	0.10	23	0.076	219.3	100	10	1	17	84
12	27	0.10	23	0.051	33.5	6.8	10	2	30	80
13	27	0.10	23	0.076	219.3	33.3	30	2	39	83
14	27	0.10	23	0.025	98.7	1.7	30	3	43	83
15	27	0.10	23	0.025	98.7	0.8	60	2	41	83
16	27	0.10	23	0.025	33.5	1.1	60	5	67	84

Valves V_1, V_2, and V_3 were configured to second positions (as shown in Figure S2). In this position (the second position of V_1 and V_3), V_1 and V_3 connect ports 1 and 2, 3 and 4, 5 and 6, 7 and 8, and 9 and 10 of the respective valves. Valve V_2 connects ports 1 and 4, and 2 and 3 in this position (the first position of V_2). Flow-path between port 3 of V_2 and port 1 of V_1 and loop L_2 were filled with nitrogen gas from pumps P_5 and P_6, respectively. At the same time, L_1 was filled with reagents and DMSO from pumps P_1 to P_4. The mixture of reagents and DMSO in L_1 gave a reaction plug representing a reaction stoichiometry under trial.

Valves V_1, V_2, and V_3 were configured back to first positions (Figure S1). Pump P_5 moved the reaction plug, which was sandwiched between two nitrogen gas plugs, to loop L_3 via reactor R, which was set at a specific temperature. Heater H was set to heat SS beads to a specific temperature for 30 min prior to introducing the reaction plug in the reaction zone. Also during this time, pump P_9, which was equipped with a 0.05 M solution of 2-methoxy naphthalene (internal standard for analysis), filled loop L_4.

Valves V_1, V_2, and V_3 were configured back to second positions (Figure S2). Pumps P_1, P_2, P_3, and P_4 filled up loop L_1 with a new mixture of reagents and DMSO representing a new reaction stoichiometry for another trial run. Pumps P_5 and P_6 filled up respective flow-paths with nitrogen gas for the second run. Pump P_8 moved the reaction specimen from the first trial run (from loop L_3) along with a fixed volume of the internal standard solution (from loop L_4) to a sampling vial (S) using N (in mode B; Figure S1) using acetonitrile as a transport fluid. Specifically, first 1100 μL of the transport fluid between port 6 of V_3 and N was discarded and next 1500 μL fluid was collected in S. This 1500 μL acetonitrile sample was used as the injectable for LC analysis. The robotic arm of LHR was moved to the cleaning station (not shown) and additional 1500 μL of acetonitrile was pumped through the flow-paths in question to ensure that the transfer tube remained clean and primed for next sampling.

A 4 μL sample of the injectable was injected into loop L_5 of V_I by LHR from the load configuration of V_I (Figure S1). Valve V_I was moved to the inject configuration (Figure S2). Valves V_{SS} and V_{HC} were configured to move the injectable in L_5 of V_I to M_1 using appropriate mobile phase (eluent) from pump P_{LC} (Figure S2). The chromatographed injectable moved downstream of M_1 and chromatographed entities pass through detector D, which registered (l=278 nm) individual entities as chromatographic peaks. Valve V_{HC} was rotated appropriately to isolate a portion of the eluent representing chromatographic peak of 8.7 min in HL_1. The remaining portion of the eluent was flowed through M_1 for another 2.3 min to complete the first-dimension portion of the multidimensional chromatographic run in 10 min (Figure S5). Valve V_{HC} was appropriately rotated again to move the isolated portion of HL_1 through M_1 for a second time. Chromatographic peak at 19.6 min represented the portion passing through D for a second time. The chromatogram bearing this peak constitutes the second-dimension portion of the multidimensional chromatogram. A portion of the eluent representing the peak from the second-dimension portion of the multidimensional chromatogram was isolated in loop HL_2. The portion was kept in HL_2 for another 3.6 min, after which V_{SS} and V_{HC} were appropriately configured to move the portion from HL_2 to M_2, which resolved the portion into both enantiomers. Detector D registered the enantiomers in the third-dimension portion of the multidimensional chromatogram. Peak at 38.1 min representing the first of the two enantiomers was recaptured in loop HL_2 and sent back to M_2 to give the fourth-dimension portion of the multidimensional chromatogram. Mobile phase for the Aldol reaction experiment (Scheme S1) was a 70:30 isocratic mixture of hexane:2-propanol at a net flow-rate of 0.75 mL/min.

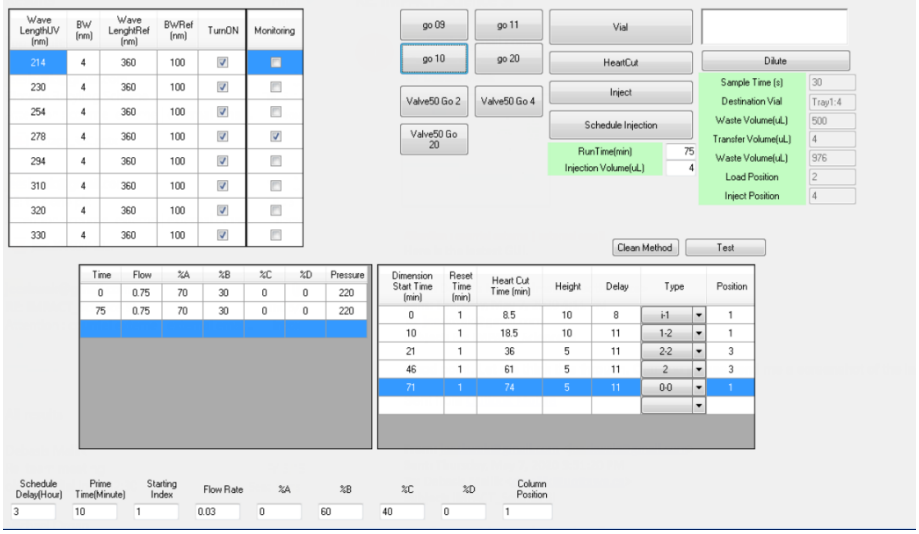


Figure S4. Screenshot of graphical user interface (GUI) running IMPACT.

Sub-window in top-left corner allows end-user to set parameters for detector D. Top-right fields of GUI allow end-users to choose post-reaction auto-sampling parameters. Bottom-left sub-window is used for setting LC methods. Bottom-right sub-window is used for setting parameters for valve V_{HC} configurations. The underlying software uses set parameters as a guideline to intelligently identify target analyte peaks that are to be isolated in a heart-cut loop and injected later from the loop. For example, row 1 of the heart-cut method window indicates that the first-dimension run (type: **i**njection to M_1; column#6 of GUI) starts at t=0 min (column#1 of GUI). Valve V_{HC} moves after t=8.5 min (column#3 of GUI) to isolate a portion of the eluent in a heart-cut loop based on hard-coded sequence (shown in top-middle portion of the GUI). The actuation of V_{HC} takes place when detector D registers the first peak after 8.5 min of elution time with a peak height of 10 units or greater (column#4 of GUI). The software reconfigures V_{HC} to move to the next position after another 8 seconds (column#5 of GUI) using path 1 of valve V_{SS} (in other words, port 1 and 1' are in fluid communication with P_{LC}; column#7 of GUI). When valve V_{HC} is re-actuated to divert the isolated portion to M_1 or M_2, valve V_{HC} stays in the re-actuated position (diverting position) for 1 min (column#2 of GUI).

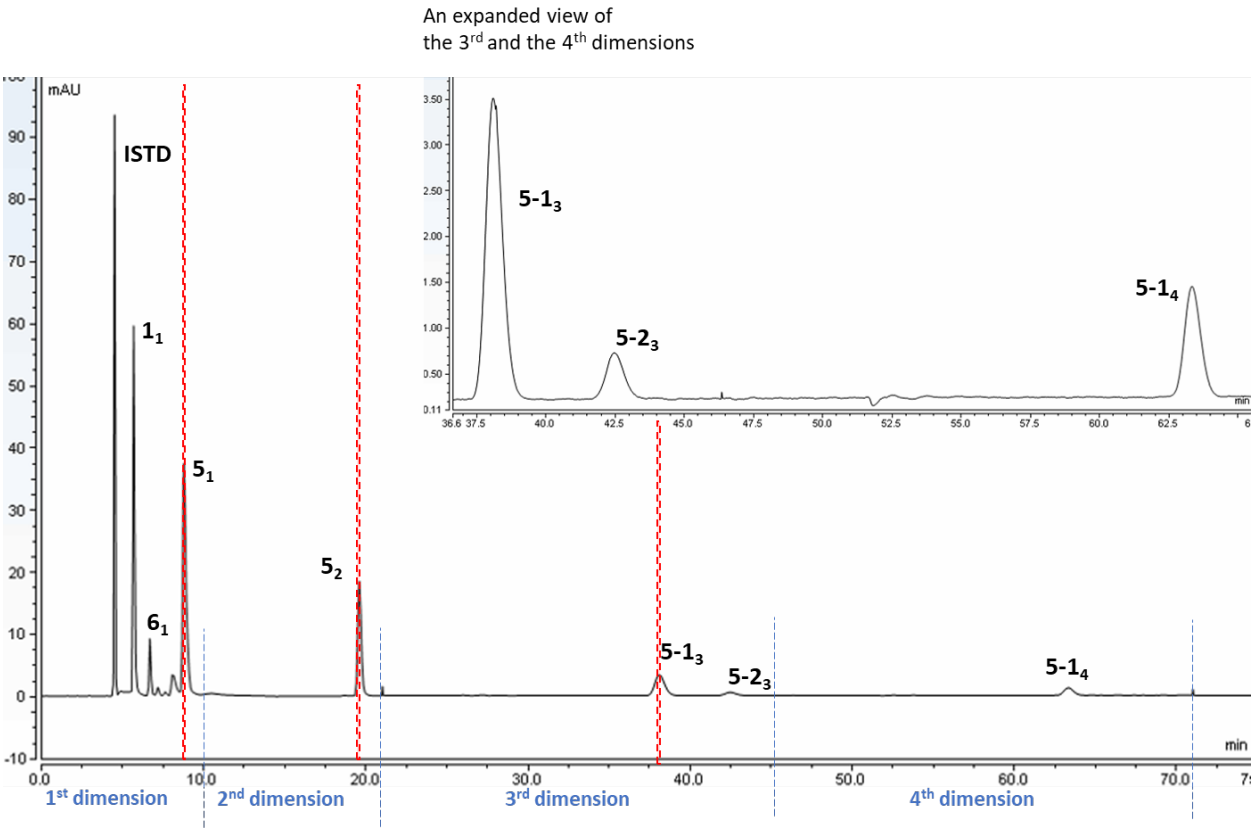


Figure S5. An example chromatogram from IMPACT analysis of model reaction (Scheme S1). Enantiomers **5a** and **5b** appear as 5-1_3 and 5-2_3 in the chromatogram; IMPACT is not currently equipped with a detector that can individually assign stereochemical structure of **5a** or **5b** to peak 5-1_3 or 5-2_3. 2-methoxynaphthalene was used as internal standard (ISTD). The heart-cutting events were indicated with red boxes in the chromatogram.

All actions from injection of a reaction specimen into V_I onward are tabulated below (Table S3).

Table S3. Sequence of events to form chromatogram of Figure S5B.

Time (min)	Action
Equilibration	P_{LC}: 0.75 mL/min hexanes:2-propanol (70:30)
	V_I: inject
	V_{SS}: C-1 (1-1')
	V_{HC}: C-1
	D: λ = 278 nm
Inject preparation	Needle mode: A
	Needle moves to wash station on LHR; wash needle exterior
	Needle moves to waste station on LHR; Needle mode: B; wash needle interior
	Needle mode: A
	Needle moves to home position
Inject sample	V_{HC}: C-9
	Needle moves to sample vial on LHR; aspirate 4 μL;
0 min	Needle moves to port 1 of V_I; V_I: load;
	Needle dispenses 4 μL; V_I:inject (initiation of first dimension)
	Needle moves to wash station on LHR; wash needle exterior
	Needle moves to waste station on LHR; needle mode: B; wash needle interior
	Needle mode: A
	Needle moves to home position

8.8 min	V_{HC}: C-10 (trapped for 9 seconds)
10 min	V_{HC}: C-11 (initiation of second dimension)
19.65 min	V_{HC}: C-9 (trapped for 12 seconds)
21 min	V_{HC}: C-3 (3-3') (initiation of third dimension)
22 min	V_{HC}: C-11
38.2 min	V_{HC}: C-20 (trapped for 13 seconds)
46 min	V_{HC}: C-9 (initiation of fourth dimension)
71 min	V_{SS}: C-1 (1-1')
75 min	End of chromatogram

Product **6** (Scheme S1) was prepared in-house for chromatographic referencing and detector calibration purposes (Figure S6). Internal standard 2-methoxy naphthalene (99 %) was purchased from Sigma Aldrich Chemical Company. Impurity standard 4-(p-nitrophenyl)-3-buten-2-one (99 %) was purchased from Toronto Research Chemicals Inc. and was used without further purification.

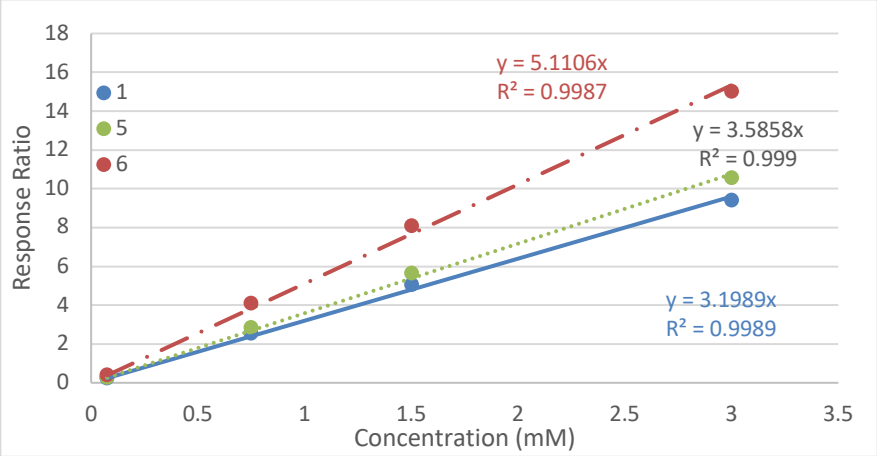


Figure S6. Internal calibration curves for 4-nitrobenzaldehyde (**1**), 4-hydroxy-4-(p-nitrophenyl)-butan-2-one (**5a**/**5b**), and 4-(p-nitrophenyl)-3-butene-2-one (**6**) are shown. Concentration of analytes were calculated from response ratio values, which were obtained from the chromatogram, according to the equation below:

$$Response\ ratio = \frac{area\ under\ analyte}{area\ under\ the\ internal\ standard}$$

Oxazepam Racemization Study.

A methanol solution of racemic oxazepam (Figure S7) was injected into V_I to conduct this study. In summary, both enantiomers were separated using a Lux 5 μm Cellulose-2 LC column (250 x 4.6 mm; Phenomenex) and individual enantiomers were chromatographed through a Luna 5 μm Silica LC column (100 x 4.6 mm; Phenomenex). Downstream ends of columns M_1 and M_2 were connected to a twenty-position, ten-port valve (specifically, ports 8 and 4 of valve V_{HC}, respectively; VICI), which was equipped with two heart-cut loops (HL_1 and HL_2; SS; 0.03" ID; 1000 μL for both; VICI). The chromatographed portion was re-circulated through the Cellulose-2 LC column for a second time to assess impact on the stability of individual enantiomers during analysis. Figure S8 shows relevant IMPACT chromatograms for this study.

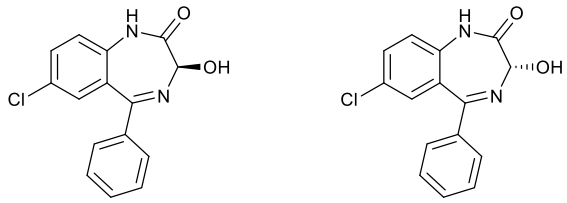


Figure S7. Enantiomers of oxazepam.

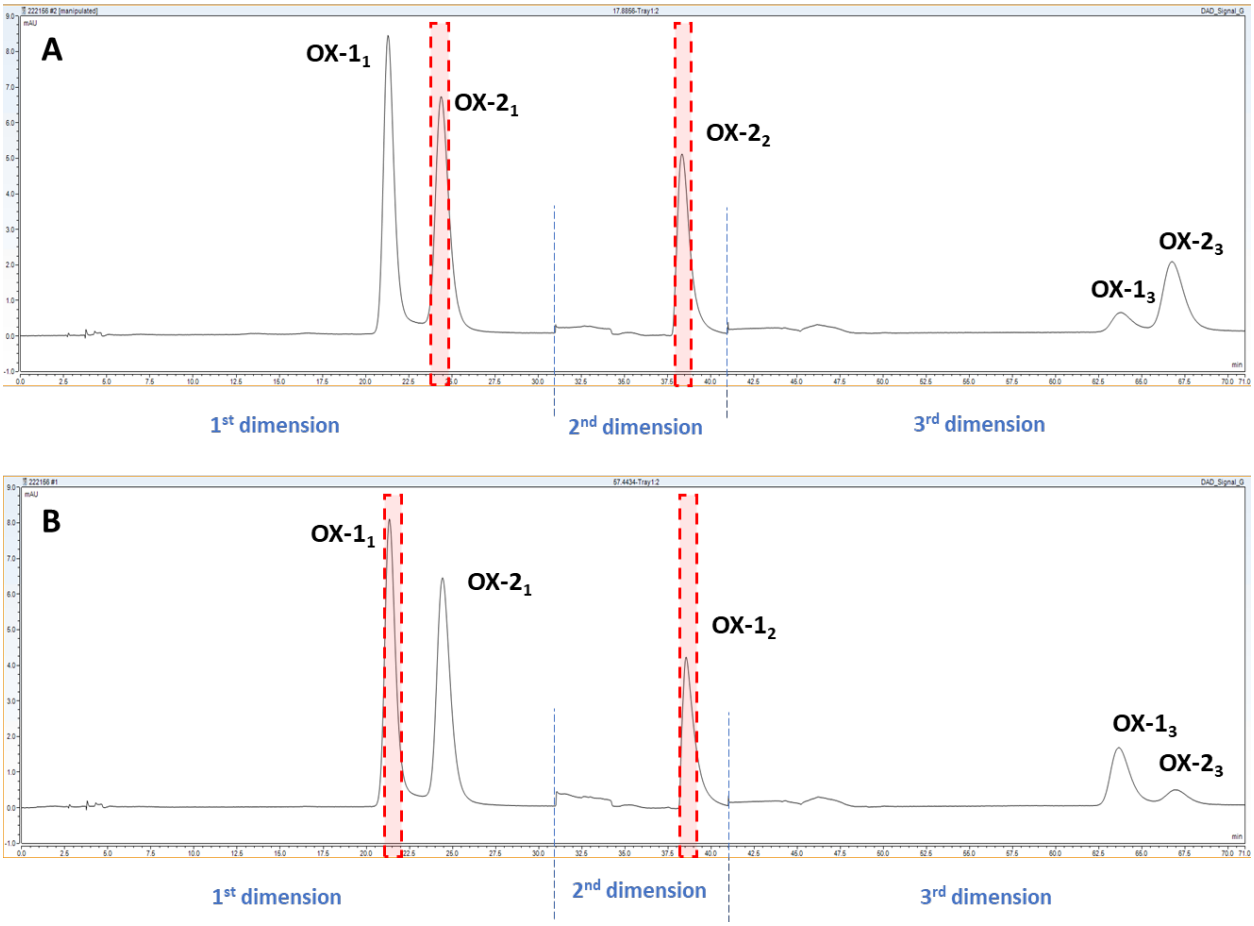


Figure S8. IMPACT analysis on oxazepam (R-OX/S-OX). A racemic mixture of oxazepam was injected into a chiral column first (the first dimension) to observe peak OX-1_1 and OX-2_1. The eluent carrying one of the pure enantiomers (OX-2_1 in Figure S8A and OX-1_1 in Figure S8B) is re-chromatographed first through an achiral column (the second dimension) to observe OX-2_2 in Figure S8A and OX-1_2 in Figure S8B and then through the chiral column (the third dimension) to assess on-column stability of oxazepam enantiomers in the achiral column. The heart-cutting events were indicated with red boxes in the chromatograms.

Analysis Setup (Oxazepam).

Ports 1' and 3' of valve V_{SS} were connected to a Luna 5 μm Silica LC column (M_1; 100 x 4.6 mm; Phenomenex) and a Lux 5 μm Cellulose-2 LC column (M_2; 250 x 4.6 mm; Phenomenex), respectively (Figure S1). Mobile phase for the racemization study of oxazepam was a 80:20 isocratic mixture of hexane:ethanol with 0.1% diethylamine in each at a net flow-rate of 1 mL/min. Racemic Oxazepam samples were placed in sampling vials of LHR.

Operating Procedure.

A 4 μL of the injectable in S was injected into loop L_5 of V_I from the load configuration of V_I (Figure S1). Valve V_I was moved to the inject configuration (Figure S2). Valves V_{SS} and V_{HC} were configured to move the injectable in L_5 of V_I to M_2 using appropriate mobile phase (eluent) from pump P_{LC} (Figure S2). The chromatographed injectable moved downstream of M_2 and chromatographed entities pass through detector D, which registered (l=320 nm) individual entities as chromatographic peaks. Valve V_{HC} was rotated appropriately to isolate a portion of the eluent representing one of the two enantiomeric peaks (OX-1 or OX-2) in HL_1. The remaining portion of the eluent was flowed through M_2. Valve V_{HC} was appropriately rotated again to move the isolated portion of HL_1 through M_1. A portion of the eluent representing peak OX-1 or OX-2 was isolated in loop HL_2. The portion was kept in HL_2 until the run through M_1 is complete, after which V_{SS} and V_{HC} were appropriately configured to move the portion of HL_2 to M_2 for a second time. Detector D registered the enantiomers in the third-dimension portion of the multidimensional chromatogram. All actions from injection of the sample into V_I onward were tabulated below (Table S4).

Table S4. Sequence of events to form chromatogram of Figure S8C.

Time (min)	Action
Equilibration	P_{LC}: 1.00 mL/min hexanes (0.1% diethylamine):ethanol (0.1% diethylamine) (80:20)
	V_I: inject
	V_{SS}: C-3 (3-3')
	V_{HC}: C-1
	D: λ = 320 nm
Inject preparation	Needle mode: A
	Needle moves to wash station on LHR; wash needle exterior
	Needle moves to waste station on LHR; Needle mode: B; wash needle interior
	Needle mode: A
	Needle moves to home position
Inject sample	V_{HC}: C-9
	Needle moves to sample vial on LHR; aspirate 4 μL;
0 min	Needle moves to port 1 of V_I; V_I: load;
	Needle dispenses 4 μL; V_I: inject (initiation of first dimension)
	Needle moves to wash station on LHR; wash needle exterior
	Needle moves to waste station on LHR; needle mode: B; wash needle interior
	Needle mode: A
	Needle moves to home position
22.1 min	V_{HC}: C-11 (trapped for 60 seconds)
31 min	V_{SS}:C-1 (1-1') (initiation of second dimension)
39.1 min	V_{HC}:C-9 (trapped for 60 seconds)
41 min	V_{SS}: C-3 (3-3') (initiation of third dimension)
71 min	End of chromatogram