Electronic Supplementary Information

High-Throughput Chiral Analysis Using Automated Desorption Electrospray Ionization Mass Spectrometry

Jie Li,[a,b] Nicolás M. Morato,[c] Bo Hao,[a] Ainslie Chen,[b] Lucile Jouffroy,[d] Ho Kim,[a] Zhicai Shi,[a] Karel Goossens,[d] Iulia I. Strambeanu,*[a] R. Graham Cooks*[b,c]

[a] Chemistry Capabilities, Analytical and Purification, Global Discovery Chemistry, Johnson & Johnson, Spring House, PA 19477 USA
[b] Department of Chemistry, Purdue University, West Lafayette, IN 47907 USA
[c] Purdue Institute for Cancer Research, Purdue University, West Lafayette, IN 47907 USA
[d] Chemical Process Research and Development, Therapeutics Development & Supply, Johnson & Johnson, Beerse, Belgium

Table of Contents

EXPERIMENTAL SECTION

Materials and reagents

2-(5-BromoBromo-2,3-dihydrobenzofuran-7-yl)piperazine (***1***) was purchased from WuXi AppTec (Shanghai, China). 1-(5-Bromopyridin-2-yl)ethan-1-ol (***4***) was purchased from BLD Pharmatech (Telangana, India). *tert*-butyl (2-hydroxycyclopentyl)carbamate (***10***) was purchased from Combi-Blocks (San Diego, CA, USA). Cobalt(II) chloride was from J.T. Baker (Radnor, PA, USA). Nickel(II) chloride and water were obtained from Thermo Fisher (Waltham, MA, USA). L-histidine was from Cyclo Industries (Jupiter, FL, USA). *O*-(1,1,1-Trifluoro-2-methylpropan-2-yl)serine (***3***), 2-((5*S*)-1-(*tert*-butoxycarbonyl)-5-(methoxy carbonyl)pyrrolidin-2-yl)acetic acid (***9***), and 2-(4-chloro-2-methoxyphenyl)-2-((3-methoxy-5-(methylsulfonyl)phenyl)amino)-1-(5-(trifluoromethoxy)-1*H*-indol-3-yl)ethan-1-one (***11***) were synthesized in the Johnson & Johnson labs. High-purity methanol and water (Riedel-de Haën CHROMASOLV LC-MS grade) were purchased from Honeywell (Morris Plains, NJ, USA). All other reagents were from Sigma Aldrich (St. Louis, MO, USA). All reagents were reagent grade or higher if not specified.

DESI plates were prepared in-house by adhering a porous PTFE membrane (Zitex G115; Saint-Gobain, Malvern, PA, USA) to soda lime glass slides (Abrisa Technologies, Santa Paula, CA, USA) using a low VOC spray adhesive (Repositionable Spray Mount; 3M, Saint Paul, MN, USA). The plates have a footprint identical to that of a standard microplate to facilitate their use in automated workflows.

Sample solution preparation

Analytes, chiral reference ligands (Ref*), and metal ions (M^{II}) were dissolved in 50% methanol/water to prepare 50 mM stock solutions and mixed at a 4:4:1 ratio, respectively, to obtain 10 mM samples in 80% methanol/water for chiral analysis. Analyte samples with diverse *ee%* were prepared by appropriately mixing different volumes (v) equimolar solutions of either commercial enantiomerically pure standards or mixtures with known *ee%* values following the equation below. These sample solutions were dispensed in 384-well polypropylene plates (Greiner Bio-One, Monroe, NC, USA) until analysis by HT-DESI-MS.

$$ee\%_x = [(v_1)\,(ee\%_1) + (v_2)\,(ee\%_2)] \,/\, (v_1 + v_2)$$

HT-DESI-MS(/MS)

The first-generation HT-DESI-MS platform, which has been described in detail[1, 2], was used for chiral analysis. The workflow followed in this study is briefly detailed below.

All experiments started by sample preparation in well plates followed by high-density array generation. This is carried out by transferring small aliquots (down to 50 nL) of samples on 384-well plates onto DESI plates (up to 6,144 samples per plate, i.e., up to sixteen 384-well plates consolidated by offsetting the transfer positions) using a 384-pin tool (V&P Scientific, San Diego, CA) compatible with the automated fluid handling workstation (Biomek i7, Beckman Coulter, Brea, CA). The pin tool is equipped with 50-nL slotted floating pins calibrated for liquid-to-liquid transfers. We optimized this array generation process by increasing the amount of material deposited so that enough analytes were on the surface for successful analysis to be achieved. Optimization involved assessing a variety of solvent matrices for the samples as well as evaluation of what number of sequential spotting operations (i.e. automated spotting of each sample over again on the same position it was already spotted) were required for an efficient transfer of material.

After optimization, we ended up with 8:2 methanol-water samples spotted 50 times, for an estimated total of 2.5 μL deposited (2.5×10^{-8} mol; ~10 μg). Note that four experimental replicates (i.e., samples corresponding to the same original wells but spotted in four different positions within the high-density array) were spotted for each sample, generating arrays of 1,536 spots per DESI plate.

Automated screening of the spotted plates at a throughput of ca. 1 spot/s then took place using a 2D DESI stage (Prosolia, Indianapolis, IN, USA) coupled to a linear ion trap mass spectrometer (LTQ XL; Thermo Fisher, San Jose, CA, USA). DESI was carried out using methanol/water (9:1) as spray solvent, which was supplied at a rate of 3 μL/min using a Harvard Apparatus syringe pump (Holliston, MA, USA), and nitrogen as nebulizing gas (150 psi). Analysis was carried out in all cases in the positive ion mode, setting the DESI voltage at 5 kV and the source temperature at 150 °C. All MS/MS experiments were performed using collision-induced dissociation (CID) with an isolation window width of 6 Da centered on the $[M^{II}(Anal)(Ref^*)_2\text{-}H]^+$ precursor ion, and with the collision energy set to 25 normalized manufacturer units. We evaluate different throughputs for this MS/MS experiment (which are typically lengthier than those using single-stage MS acquisition) and found an analysis rate of 12 s per sample sufficient for high quality data without a significant compromise of speed. The automated HT-DESI-MS(/MS) analysis and integrated data processing were carried out using a combination of Python- and MATLAB-based custom software, respectively.

Data analysis

Average single-stage MS intensities across experimental replicates were used to calculate signal-to-noise ratios (SNR) for ions of interest, i.e., *m/z* values corresponding to expected trimeric complex ions $[M^{II}(Anal)(Ref^*)_2\text{-}H]^+$. For each set of analyte/Ref*/M^{II}, successful hits were defined as those with SNR values higher than 3 and at least 200 average ion counts. Only successful hits were subjected to MS/MS experiments to confirm the identification and adequacy of the detected complex based on the observation of the competitive loss of both analyte and Ref*. MS/MS files were processed to extract ion intensities of the fragments of interest on each spot, followed by averaging across experimental replicates. This MS/MS confirmation allowed selection of optimal Ref* and M^{II} for *ee*% determinations of each analyte.

HT-DESI-MS/MS ee% determinations

To establish the calibration curves for *ee*% determination, analyte standards or mixtures with known *ee*% were mixed with optimal Ref* and M^{II} at a ratio of 4:4:1 to obtain 10 mM standards in 80% methanol/water. Unknown samples were prepared similarly by the addition of Ref* and M^{II}. All samples were subjected to HT-DESI-MS/MS analysis as described above. Extracted ion intensities of the fragments of interest were used to calculate ln(*R*) values for each sample as described below.

RELEVANT EQUATIONS AND DEFINITIONS

The kinetic method derivation and implementation have been described in detail in previous reports.[3-7] Here, some relevant definitions and equations are summarized.

The ratio of the rates of the two competitive fragmentation pathways (R) is defined as:

$$R = k_A/k_{ref} = [M^{II}(Analyte)(Ref^*) - H]^+/[M^{II}(Ref^*)_2 - H]^+$$

The natural logarithm of this ratio is proportional to the difference between the M^{II} affinities of the two dimeric fragment complexes under the previously described assumption of identical frequency factors for the competing dissociations, meaning that the entropy terms for the reactions are equal.

So, using M^{II}BDE for metal ion affinity, i.e. the negative of the enthalpy change for the binding of the analyte to the metal ion M^{II}, one has:

$$\ln(R) = [(\Delta M^{II}BDE[(Analyte)(Ref^*) - H]^+) - (\Delta M^{II}BDE[(Ref^*)_2 - H]^+)]/\mathbf{R}T_{eff}$$

Note that **R** is the gas constant. The energy change terms are simply enthalpy changes for the reactions:

$$Analyte + Ref^* + M^{II} \rightarrow [M^{II}(Analyte)(Ref^*) - H]^+ + H^+ \qquad (\Delta M^{II}BDE[(Analyte)(Ref^*)\text{-}H]^+)$$

$$2Ref^* + M^{II} \rightarrow [M^{II}(Ref^*)_2 - H]^+ + H^+ \qquad (\Delta M^{II}BDE[(Ref^*)_2\text{-}H]^+)$$

Chiral selectivity (R_{chiral}) is defined as the ratio of R_D/R_L, where:

$$R_D = [M^{II}(Analyte)_D(Ref^*) - H]^+/[M^{II}(Ref^*)_2\text{-}H]^+$$

$$R_L = [M^{II}(Analyte)_L(Ref^*) - H]^+/[M^{II}(Ref^*)_2\text{-}H]^+$$

The difference in M^{II} affinities between the D and L forms of the analyte is then proportional to R_{chiral}:

$$\Delta(\Delta M^{II}BDE) = (\Delta M^{II}BDE[(Analyte)_D(Ref^*) - H]^+) - (\Delta M^{II}BDE[(Analyte)_L(Ref^*) - H]^+)$$

$$\Delta(\Delta M^{II}BDE) = \mathbf{R}T_{eff}\ln(R_{chiral})$$

SUPPLEMENTARY FIGURES AND TABLES

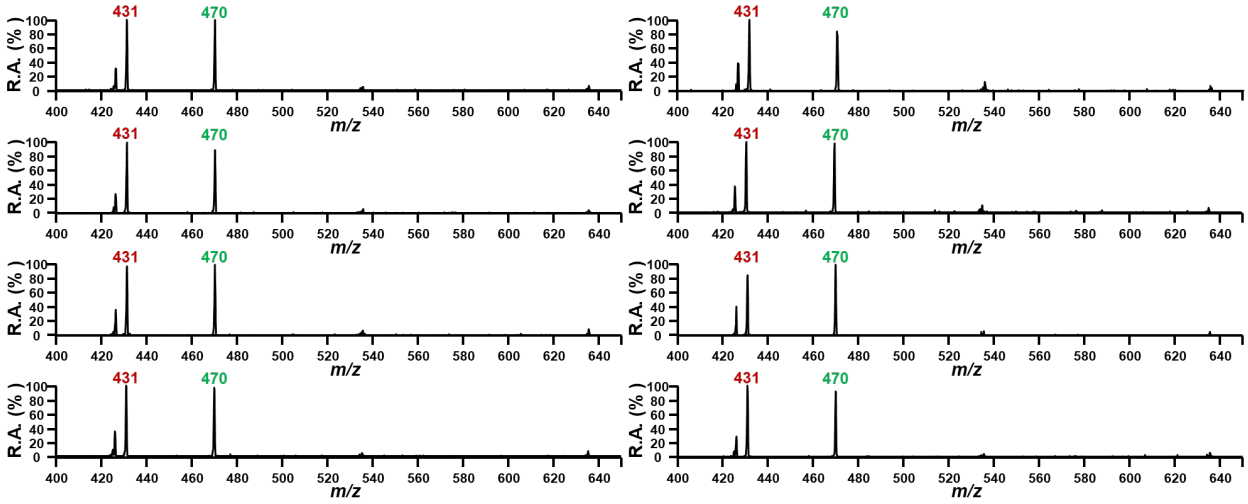

Figure S1. Example MS/MS spectra (8 technical replicates) obtained during chiral analysis of ephedrine (–100% *ee*) using HT-DESI-MS/MS (12 s/sample). The chiral reference compound is *L*-tryptophan, and the metal ion is Cu^{2+}.

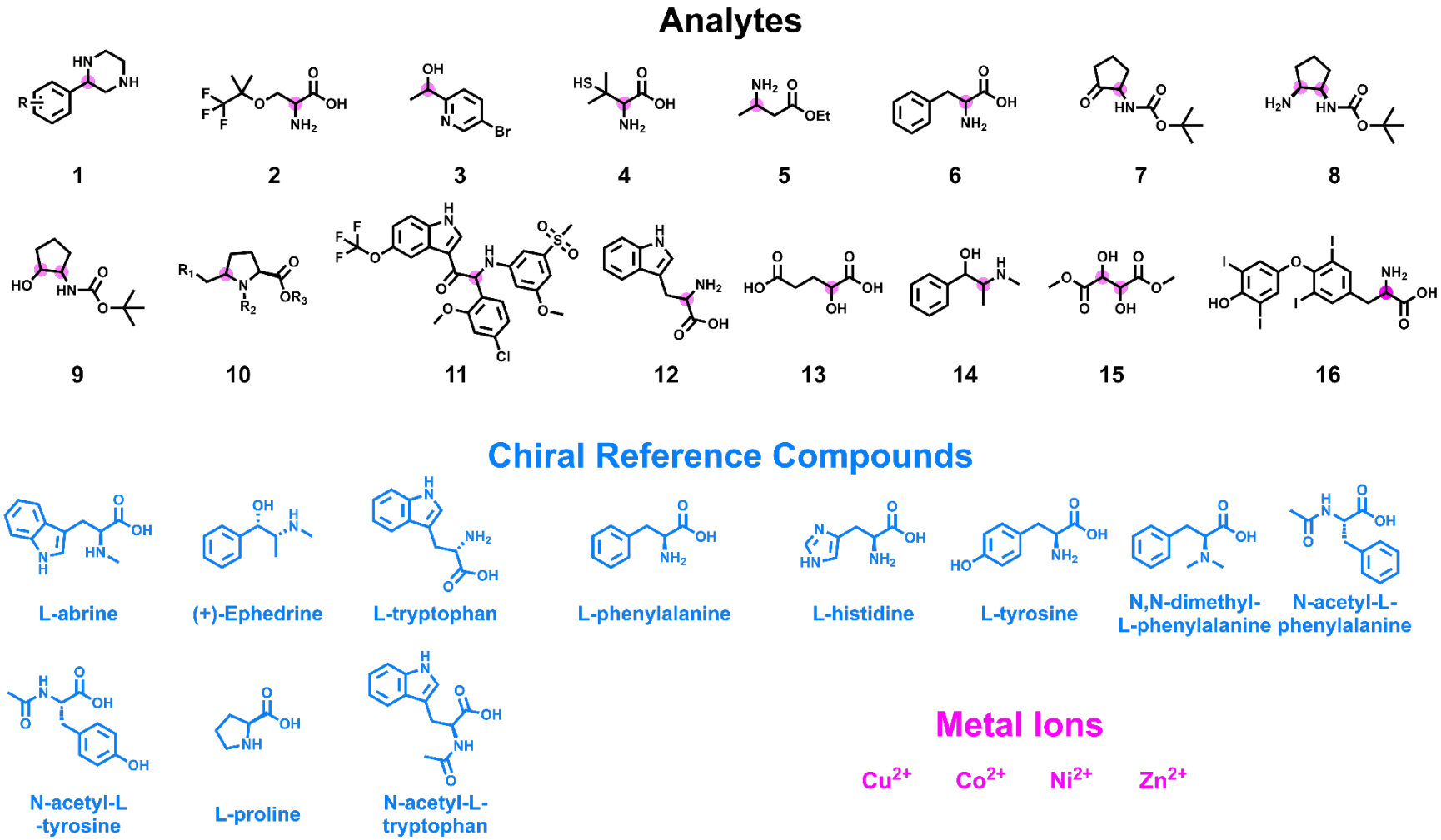

Figure S2. Structures of the 16 analytes subjected to HT-DESI-MS during chiral method development by screening combinatorial pairs of the 11 chiral reference compounds and 4 metal ions shown.

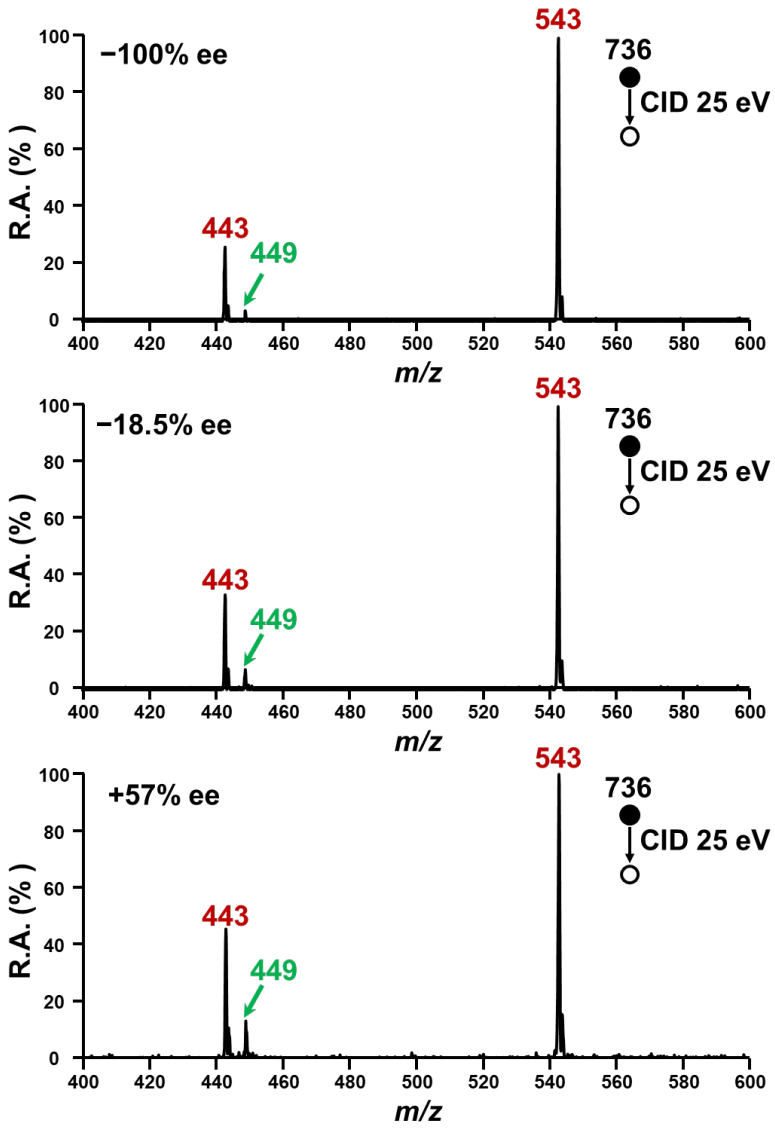

Figure S3. Representative MS/MS spectra obtained during chiral analysis of organic synthesis product ***10*** using HT-DESI-MS/MS (12 s/sample) at different *ee*% values. From top to bottom: –100% *ee*, –18.5% *ee*, and +57% *ee* (*ee*% measured by LC). The chiral reference compound is *N*,*N*-dimethyl-L-phenylalanine and the metal ion is Zn^{2+}.

Table S1. HT-DESI-MS *ee*% determination of ephedrine standards with known *ee*% values.

Standards	Nominal *ee*% values (%)	HT determined *ee*% values (%)
1	**87.5**	**88.4 ± 7.8**
2	**27.5**	**28.9 ± 3.1**
3	**–26.0**	**–23.4 ± 3.0**
4	**–86.0**	**–83.8 ± 6.2**

Table S2. Applicability of chiral reference ligand/metal ion pairs for kinetic method *ee*% determination of 16 compounds by HT-DESI-MS (1 sample/s) (see **Figure S2**). Green represents likely hits as judged by signal intensity (ion count > 200) and SNR (> 5) of the expected *m/z* values corresponding to the deprotonated trimeric complexes. Red denotes low probability hits (5 ≥ SNR > 3). Triads not shown in the matrix yielded no detectable ions for the expected complexes.

Chiral reference	Analyte															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
L-abrine		Zn	Zn Cu				Zn Ni Cu	Ni	Ni					Cu		
(+)-Ephedrine											Ni					
L-tryptophan		Cu	Ni Zn				Ni Cu		Co Cu							Co
L-phenylalanine		Zn Ni	Co Cu			Co			Zn							
L-histidine	Ni			Ni				Ni		Ni						
L-tyrosine																Ni Cu
N,N-dimethyl-*L*-phenylalanine	Cu	Co Zn Cu		Ni		Ni	Ni Cu	Cu Ni Zn		Co Zn		Co Zn				
N-acetyl-L-phenylalanine			Ni				Co		Co Zn				Zn			
N-acetyl-L-tyrosine						Ni			Co							
L-proline		Zn Ni						Co				Co Zn	Cu			Co
N-acetyl-L-tryptophan	Cu															

Table S3. HT DESI-MS *ee*% determinations of organic synthesis product ***10*** from reaction mixtures, compared with *ee*% values determined by LC-UV.

Samples	LC-UV determined *ee*% values (%)	HT-DESI-MS determined *ee*% values (%)
1	**41.9**	**40.8 ± 3.2**
2	**4.2**	**3.8 ± 0.4**
3	**–22.3**	**–25.4 ± 4.1**
4	**–52.5**	**–54.9 ± 1.7**
5	**–71.4**	**–71.8 ± 4.7**
6	**–94.0**	**–97.6 ± 8.9**

REFERENCES

1. T. J. P. Sobreira, L. Avramova, B. Szilagyi, D. L. Logsdon, B. P. Loren, Z. Jaman, R. T. Hilger, R. S. Hosler, C. R. Ferreira, A. Koswara, D. H. Thompson, R. G. Cooks and Z. K. Nagy, *Analytical Methods*, 2020, **12**, 3654-3669.
2. N. M. Morato, M. T. Le, D. T. Holden and R. G. Cooks, *SLAS Technol.*, 2021, **26**, 555-571.
3. R. G. Cooks and P. S. H. Wong, *Accounts Chem. Res.*, 1998, **31**, 379-386.
4. W. A. Tao, D. Zhang, F. Wang, P. D. Thomas and R. G. Cooks, *Anal. Chem.*, 1999, **71**, 4427-4429.
5. W. A. Tao, D. X. Zhang, E. N. Nikolaev and R. G. Cooks, *J. Am. Chem. Soc.*, 2000, **122**, 10598-10609.
6. W. A. Tao, F. C. Gozzo and R. G. Cooks, *Anal. Chem.*, 2001, **73**, 1692-1698.
7. L. M. Wu and R. G. Cooks, *Anal. Chem.*, 2003, **75**, 678-684.