Electronic Supplementary Material (ESI) for Analytical Methods.

ELECTRONIC SUPPLEMENTARY INFORMATION

Easy Protocol for Making a Good *E*-Sinapinic Acid Matrix for Neutral and Sulfated Carbohydrate MALDI-MS Analysis

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EXPERIMENTAL

Instrumentation

UV/VIS Analysis. Electronic absorption spectra were recorded on a Shimadzu PC2101 spectrophotometer. Measurements were made in quartz cells of 1 cm and 0.1 cm optical-path length. The absorption spectra were recorded in MeOH or MeCN solutions at 298 K. Wavelength maxima are reported in nm.

NMR Analysis. The ^{1}H NMR spectra were recorded at 200 MHz on a Bruker AC-200 spectrometer in DMSO solution. ^{1}H and ^{13}C NMR mono- and bidimensional spectra were carried out on a Bruker AVANCE II 500 NMR spectrometer operating at 500.14 and 125.76 MHz for ^{1}H and ^{13}C, using DMSO-d6 ($(CD_3)_2SO$) as solvent. Tetramethyl silane was used as the internal standard. ^{1}H-^{13}C heteronuclear chemical shift correlation spectrum (HSQC) was recoded using the standard pulse sequence. A total of 32 scans were accumulated with a relaxation delay of 2 s for each of the 512 t1 experiments. Chemical shift values are reported in ppm and the coupling constants are give in Hertz.

Z- (cis-) and E (trans)-3-phenyl-2-propenoic (cinnamic) acid ($C_6H_5CH=CHCOOH$) have different $J_{H,H}$ values for the coupling between the protons attached to the double bond carbons. The coupling constant of *Z* protons is generally smaller (7-12 Hz) than that of *E* protons (13-30 Hz.

Characteristics of the investigated matrices

Characterization of the irradiated E-cinnamic acids solutions

***Z*-SA (*ciZ*-4-Hydroxy-3,5-dimethoxycinnamic acid; *Z*-sinapinic acid)** [1].

m.p.; ^{13}C NMR; UV-vis absorption spectrum; EI MS (70 eV); HRMS negative ion mode; HRMS MS/MS negative ion mode were described elsewhere[1, 2, 3].

^{1}H NMR (200 MHz, $(CD_3)_2SO$) □ 3.75 (s, 6H, OCH_3), 5.76 (d, J = 12.8 Hz, 1H, H□), 6.76 (d, J = 12.8 Hz, 1H, H□), 7.23 (s, $HC_{2\text{-}6}$), 8.88 (s, 1H, OH).

***E*-SA (*E*-4-Hydroxy-3,5-dimethoxycinnamic acid; *E*-sinapinic acid)** [1].

m.p.; ^{13}C NMR; UV-vis absorption spectrum; EI MS (70 eV); HRMS negative ion mode; HRMS MS/MS negative ion mode were described elsewhere[1, 2, 3].

^{1}H NMR (200 MHz, $(CD_3)_2SO$) □: 3.80 (s, 6H, OCH_3), 6.42 (d, J = 15.8 Hz, 1H, H□), 6.99 (s, 2H, HC(2) and HC(6)), 7.50 (d, J = 16 Hz, 1H, H□).

Irradiated *E*-SA (I- *E*-SA)

^{1}H NMR (200 MHz, $(CD_3)_2SO$) □ 3.76 (s, 6H, OCH_3), 3.80 (s, 6H, OCH_3), 5.77 (d, J = 12.0 Hz, 1H, H□ [*Z*-SA]), 6.42 (d, J = 16.0 Hz, 1H, H□ [*E*- SA]), 6.76 (d, J = 12.0 Hz, 1H, H□ [*Z*- SA]), 6.99 (s, 2H, HC(2) and HC(6)), 7.23 (s, $HC_{2\text{-}6}$), 7.50 (d, J = 16 Hz, 1H, H□ [*E*- SA]) (Fig. S6c).

***E*-FA (*trans*-4-Hydroxy-3-methoxycinnamic acid; *trans*-Ferulic acid; t-FA).** S3

m.p.; ^{13}C NMR; UV-vis absorption spectrum; EI MS (70 eV); HRMS negative ion mode; HRMS MS/MS negative ion mode were described elsewhere[1, 2].
^{1}H NMR (200 MHz, $(CD_3)_2SO$) □ 3.82 (s, 3H, OCH_3), 6.37 (d, *J* = 16 Hz, 1H, H□), 6.80 (d, *J* = 8.2 Hz, 1H, HC(5)), 7.09 (dd, *J* = 8.2, 1.8 Hz, 1H, HC(6)), 7.28 (d, *J* = 1.8 Hz, 1H, HC(2)), 7.49 (d, *J* = 16 Hz, 1H, H□).

***Z*-FA (*cis*-4-Hydroxy-3-methoxycinnamic acid; *cis*-Ferulic acid; c-FA).**

m.p.; ^{13}C NMR; UV-vis absorption spectrum; EI MS (70 eV); HRMS negative ion mode; HRMS MS/MS negative ion mode were described elsewhere[1, 2].
^{1}H NMR (200 MHz, $(CD_3)_2SO$) □ 3.76 (s, 3H, OCH_3), 5.74 (d, *J* = 12.6 Hz, 1H, H□), 6.77 (dd, 2H, *J* = 12.8 Hz, H□ and *J* = 9.0 Hz, HC(5)), 7.15 (d, *J* = 7.6, 1H, HC(6)), 7.67 (s, 1H, HC(2)), 9.50 (s, 1H, OH).

Irradiated *E*-FA (I- *E*-FA)

^{1}H NMR (200 MHz, $(CD_3)_2SO$) □□3.76 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 5.74 (d, *J* = 12.0 Hz, 1H, H□ [*Z*]), 6.37 (d, *J* = 16.0 Hz, 1H, H□ [*E*]), 6.77 (m, *J* = 12.0, 8.0 Hz, 3H, H□ [*Z*], HC(5)), 7.12 (m, *J* = 8.0, 2.0 Hz, 2H, HC(6)), 7.28 (d, *J* = 2.0 Hz, 1H, HC(2)), 7.49 (d, *J* = 16 Hz, 1H, H□ [*E*]), 7.65 (d, 1H, *J* = 2.0 Hz, HC(2)) (Fig. S6b).

***E*-CuA *(trans*-4-Hydroxycinnamic acid; *trans-p*-coumaric acid; t-CuA).**

m.p.; ^{13}C NMR; UV-vis absorption spectrum; EI MS (70 eV); HRMS negative ion mode; HRMS MS/MS negative ion mode were described elsewhere[1, 2].
^{1}H NMR (200 MHz, $(CD_3)_2SO$) □ 6.29 (d, *J* = 16.0 Hz, 1H, H□), 6.79 (d, 2H, HC(2) and HC(6)), 7.49 (m, 3H, HC(3), H(5) and H□, d, *J* = 16.0 Hz), 9.98 (s, 1H, OH).

***Z*-CuA (*cis*-4-Hydroxycinnamic acid; *cis-p*-coumaric acid; c-CuA).**

m.p.; ^{13}C NMR; UV-vis absorption spectrum; EI MS (70 eV); HRMS negative ion mode; HRMS MS/MS negative ion mode were described elsewhere[1, 2].
^{1}H NMR (200 MHz, $(CD_3)_2SO$) □ 5.73 (d, *J* = 12.8 Hz, 1H, H□), 6.77 (m, 3H, HC(2), HC(6) and H□), 7.65 (d, *J* = 8.2 Hz, 2H, HC(3) and HC(5)), 9.92 (s, 1H, OH).

Irradiated *E*-CuA (I- *E*-CuA).

^{1}H NMR (200 MHz, $(CD_3)_2SO$) □□5.73 (d, *J* = 12.0 Hz, 1H, H□ [Z]), 6.29 (d, *J* = 16.0 Hz, 1H, H□ [*E*]), 6.78 (m, 5H, HC(2), HC(6) and H□ [*Z*]), 7.55 (m, 5H, HC(3), H(5) and H□ [*E*], d, *J* = 16.0 Hz) (Fig. S6a).

***E*-CAF *(trans*-3,4,-dihydroxycinnamic acid; *trans*-caffeic acid; t-CAFA).**

^{1}H NMR (200 MHz, $(CD_3)_2SO$) □ 6.18 (d, *J* = 16.0 Hz, 1H, H□), 6.77 (d, *J* = 8.0 Hz 1H, HC(5)), 7.00 (m, 2H, HC(2) and HC(6)), 7.43 (d, *J* = 16.0 Hz, 1H, H□), 9.14 (s, 1H, OH), 9.54 (s, 1H, OH).

***Z*-CAF *(cis*-3,4,-dihydroxycinnamic acid; *cis*-caffeic acid; c-CAFA).**

^{1}H NMR (200 MHz, $(CD_3)_2SO$)□□ 5.69 (d, *J* = 12.0 Hz, 1H, H□ [*Z*]), 6.17 (d, *J* = 16.0 Hz, 1H, H□ [*E*]), 6.71 (m, 3H, H□ [*Z*], HC(5) [*Z*]and HC(5) [*E*]), 7.00 (m, 3H, HC(6) [*Z*], HC(2) [*E*] and HC(6) [*E*]), 7.38(m, 2H, HC(2) [*Z*] and H□ [*E*], d, *J* = 16.0 Hz), 9.01 (s, 1H, OH [*Z*]), 9.14 (s, 1H, OH [*E*]), 9.35 (s, 1H, OH [*Z*]), 9.54 (s, 1H, OH [*E*]).

Irradiated *E*-CAF (I- *E*-CAFA) S4

^{1}H NMR (200 MHz, $(CD_3)_2SO$))□□ 5.69 (d, *J* = 12.0 Hz, 1H, H□ [*Z*]), 6.17 (d, *J* = 16.0 Hz, 1H, H□ [*E*]), 6.71 (m, 3H, H□ [*Z*], HC(5) [*Z*]and HC(5) [*E*]), 7.00 (m, 3H, HC(6) [*Z*], HC(2) [*E*] and HC(6) [*E*]), 7.38(m, 2H, HC(2) [*Z*] and H□ [*E*], d, *J* = 16.0 Hz), 9.01 (s, 1H, OH [*Z*]), 9.14 (s, 1H, OH [*E*]), 9.35 (s, 1H, OH [*Z*]), 9.54 (s, 1H, OH [*E*]).
(Fig. S6d)
Physical properties (m.p., UV-absorption spectra, ^{1}H-NMR, ^{13}C-NMR, EI-MS and HRMS (ESI-MS)) for characterization of *E*- and *Z*-cinamic acids, and *Z*-cinnamic acids synthesis have been described elsewhere[1, 2, 3]. Here is important to note that (i) in all cases the m.p. of the *E*-isomer is higher than that of the *Z*-isomer, i.e., *E*-SA 185 ^{o}C and *Z*-SA 116-118 ^{o}C[1, 2, 3]. Thus, the m.p. of the mixture E-SA+Z-SA (*aprox*. 1:1 (mol/mol)) is always higher than the m.p. of the Z-isomer, i.e., *E*-SA+*Z*-SA 1:1 (mol/mol) 110-184 ^{o}C; (ii) the UV-absorption spectra in methanol solution for the *E*- and *Z*-isomers are quite similar, i.e., □max (nm) (log □): *E*-SA 322 (4.14) and *Z*-SA 317 (4.01) (10^{-5}-10^{-4} M; Beer – Lambert conditions[4, 5]). As consequence the spectrum of *E*-acid + *Z*-acid mixtures (1:9 to 9:1) are similar too (i.e., *E*-SA + *Z*-SA □max (nm) 322 – 317). As was described elsewhere[3] *E*-SA and *Z*-SA UV absorption spectra acquired increasing matrix concentration, beyond the Beer-Lambert conditions[4, 5] showed that the bands were getting wider and wider showing a bathochromic shift (red shift). Thus, at higher concentration (10^{-3}-10^{-2} M) and as an extrapolation in solid state, *E*-SA and Z-SA absorb efficiently at longer wavelengths including 355nm[3]; the same behavior has been observed for the pre-prepared *E*-SA+Z-SA mixtures and for irradiated *E*-SA methanolic solution (I-*E*-SA) used as matrix (results not shown).
Characterization of (I-*E*-SA) was based on the ^{1}H-NMR spectrum (Fig. S6d; see detailed data above). For morphological inspection of the solid matrix deposited on the probe the dried droplet method was used to prepare the sample. Fresh matrix solution was prepared in methanol/water as detailed in Experimental. As a result, *E*-SA gave small crystals distributed at random, as aggregates, all over the sample surface and *Z*-S5

SA showed the aspect of a solid solution (glass) with few tinny heterogeneous round spots; the irradiated E-SA methanolic solution (I-*E*-SA) as well as the pre-prepared *E*-SA + *Z*-SA (1:1) mixture yielded a solid deposit with heterogeneous aspect more similar to that of *E*-SA). When solid analyte-matrix sample were prepared on the probe with *E*-SA, *Z*-SA, pre-prepared *E*-SA + *Z*-SA (1:1) mixture and (I-*E*-SA), the morphological aspect of each sample was similar to that of the matrix alone (i.e., see Fig. S7, analyte: neocarratetraose 4_1,4_3-disulfate disodium salt (NCT); matrix: (a) *E*-SA, (b) *Z*-SA and (c) (I-*E*-SA)). Digital images were registered on the Bruker Ultraflex II TOF/TOF.

MALDI mass spectrometry analysis of Matrices

Laser desorption / ionization (LDI) experiments were conducted with the 355 nm laser on the Bruker Ultraflex II TOF/TOF in positive and negative ion modes. Experiments were run in exactly the same conditions (solution concentration; solvent; volume loaded on the probe). In both ion modes the laser energy applied (laser power) was slightly above the ionization threshold and similar to that used in experiments conducted for carbohydrate analysis.

As was described elsewhere[1], in positive ion mode *Z*-SA always showed more abundant peaks than *E*-SA. The spectrum of *E*-SA showed the protonated intact molecular ion peak at m/z 225.42 $[M+H]^+$ and the predominant signal at m/z 207.37 (base peak) assigned to the species $[M-OH]^+$. as well as peaks at higher m/z corresponding to $[M+Na]^+$ (247.60) and at m/z 285.56 (not assigned). *Z*-SA showed signals at m/z 207.13 $[M-OH]^+$, 225.40 $[M+H]^+$ (small peak), 247.43 $[M+Na]^+$, 263.61 $[M+K]^+$ and signals not assigned at m/z 269.43, 285.53 (base peak) and 301.77. Irradiated *E*-SA methanolic solution (I-*E*-SA) showed abundant signals as the spectrum of *Z*-SA above described. In negative ion mode the spectra of *Z*-SA and *E*-SA were

quite simple. The [M-H]- species and the deprotonated dimeric cluster at m/z 222.40 and 446.42 for the former and at m/z 222.47 and 446.49 for the latter were observed (Figures S8A and S8B). Although they looked quite similar always the intensity of the signals corresponding to the deprotonated dimeric cluster at m/z 444 is higher in the *E*-SA than in the *Z*-SA. This characteristic is kept in the spectrum of the (I-*E*-SA) after 3-4 h irradiation but dimeric cluster ion intensity is higher than ion molecular signal if irradiation time is 8 hs (Figs. S8C and 58D), suggesting that the flat molecular structure of the *E*-SA rules the easier dimeric cluster formation among SA molecules although the presence of *Z*-SA; this process is not so efficient when only unities of *Z*-SA are present in the solid sample. Similar LDI results were obtained for the *E*-, *Z*- and irradiated-E-(I-*E*-) forms of the other cinnamic acids studied (FA, CuA and CAF; results not shown).

Molecular Modeling.

The ground state geometry of *Z*- and *E*-cinnamic acids were fully optimized without imposing any symmetry constraints by *ab initio* and semiempirical methods (Scheme 1 and Scheme S1, (a) *E*-SA and (b) *Z*- SA). For *ab initio* DFT calculations, we used the hybrid gradient-corrected exchange functional combined with the gradient-corrected correlation functional, commonly known as B3LYP which has been shown to be quite reliable for geometries. For geometries optimization the standardized 6-311G(d,p) basis set was used. We denote our B3LYP calculations by B3LYP/6-311G(d,p). For single point calculations the standarized 6-311++G(d,p) basis set was used at B3LYP theory level (B3LYP/6-311++G(d,p)//B3LYP/6-311G(d,p). All the a*b initio* calculations were carried out, using Gaussian 98W program.[6,7] S7

References

1. M. L. Salum, C. J. Robles and R. Erra-Balsells, *Org. Lett.,* 2010, 12, 4808-4811.
2. M. L. Salum and R. Erra-Balsells, *One-pot synthesis of Z-cinnamic acids***.** Argentine Patent, CONICET #20090105020, 2009.
3. M. L. Salum, L. M. Itovich and R. Erra-Balsells, *J. Mass Spectrom.,* 2013, 48, 1150-1159.
4. M. Klessinger and J. Michl, *Excited States and Photochemistry of Organic Molecules*; VCH Publishers, Inc., New York, 1995, pp. 362.
5. N. J. Turro, V. Ramamurthy, and J. C. Scaiano, *Principles of Molecular Photochemistry: An Introduction*. University Science Books, Sausalito, 2009, pp. 319.
6, Gaussian 98W, Revision A.3., Gaussian 98, Revision A.3, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery, Jr., R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, Q. K. Morokuma, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V, Ortiz, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, C. Gonzalez, M. Challacombe, P. M. Gill, B. Johnson, W. Chen, M. W. Wong, J. L. Andres, C. Gonzalez, M. Head-Gordon, E. S. Replogle, J. A. Pople, Gaussian, Inc., Pittsburgh PA, 1998.
7. HyperChem 8.08 for Windows, HyperCube Inc., Ontario, 2010. S8
h MeOH

Scheme S1. *E/Z* Photoisomerization of cinnamic acids in methanolic solution (i.e., *E/Z* photoisomerization of sinapinic acid in MeOH solution, (a) *E*-SA, (b) *Z*-SA. S9

O HOHOHO OH O HOHO OH O O O HOHO OH OH n-1 O O HO O HO O OSO3-Na+ C OH O O HO O O O OSO3-Na+ C OH OH n-1 H2 OH H2OH

Maltoses Sulfated carbohydrates

O O O HOH2C OH HO O O HOH2C OH HO O HOH2C OH O HO OO O O O O O CH2OH HO CH2OH OH OH HOH2C CH2OH OH HO HO HO HO

-Cyclodextrin

CH2OH HOH2C O OH OH CH2 HOH2C O OH OH OO O HOHO HOH2C OH n-1

Fructanes

Scheme S2. Representative molecular structure of carbohydrates studied. S10

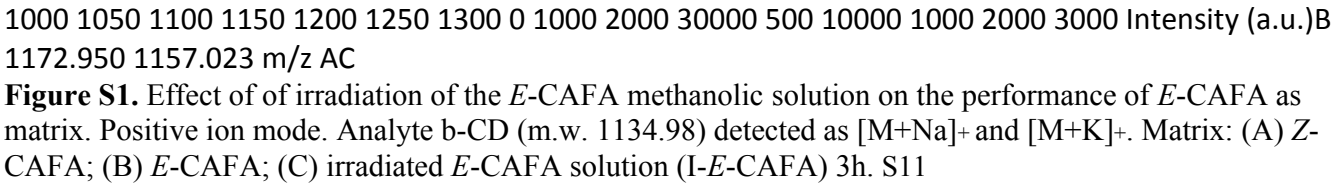


Figure S1. Effect of of irradiation of the *E*-CAFA methanolic solution on the performance of *E*-CAFA as matrix. Positive ion mode. Analyte b-CD (m.w. 1134.98) detected as $[M+Na]^+$ and $[M+K]^+$. Matrix: (A) *Z*-CAFA; (B) *E*-CAFA; (C) irradiated *E*-CAFA solution (I-*E*-CAFA) 3h. S11

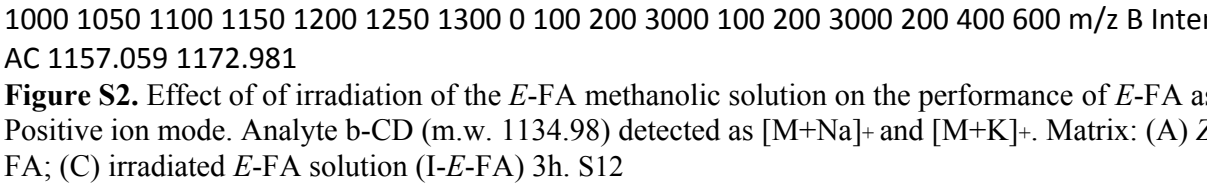


Figure S2. Effect of of irradiation of the *E*-FA methanolic solution on the performance of *E*-FA as matrix. Positive ion mode. Analyte b-CD (m.w. 1134.98) detected as $[M+Na]^+$ and $[M+K]^+$. Matrix: (A) *Z*-FA; (B) *E*-FA; (C) irradiated *E*-FA solution (I-*E*-FA) 3h. S12

Figure S3. Effect of of irradiation of the *E*-CuA methanolic solution on the performance of *E*-CuA as matrix. Positive ion mode. Analyte b-CD (m.w. 1134.98) detected as $[M+Na]_+$ and $[M+K]_+$. Matrix: (A) *Z*-CuA; (B) *E*-CuA; (C) irradiated *E*-CuA solution (I-*E*-CuA) 3h. S13

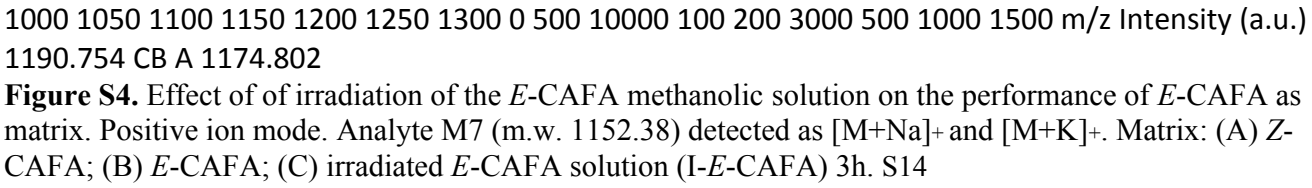


Figure S4. Effect of of irradiation of the *E*-CAFA methanolic solution on the performance of *E*-CAFA as matrix. Positive ion mode. Analyte M7 (m.w. 1152.38) detected as $[M+Na]_+$ and $[M+K]_+$. Matrix: (A) *Z*-CAFA; (B) *E*-CAFA; (C) irradiated *E*-CAFA solution (I-*E*-CAFA) 3h. S14

Figure S5. Effect of of irradiation of the *E*-CuA methanolic solution on the performance of *E*-CuA as matrix. Positive ion mode. Analyte M7 (m.w. 1152.38) detected as $[M+Na]^+$ and $[M+K]^+$. Matrix: (A) *Z*-CuA; (B) *E*-CuA; (C) irradiated *E*-CAFA solution (I-*E*-CuA) 3h. S15 S16

Figure S6. Partial 1H-NMR spectra (5-8 ppm) of "photo-make at home" matrices: (a) I-*E*-CuA, (b) I-*E*- FA, (c) I-*E*-SA and (d) I-*E* CAFA. The H□ and H□ at the alkene position (diagnosis signals) are indicated for each mixture of *E*- and *Z*- isomers. Solvent: DMSO-d6. As an example molecular structure of the isomers are indicated for I-*E*-SA in Scheme 1 and Scheme S1. S17

548 µm
548 µm
(b)
(a)

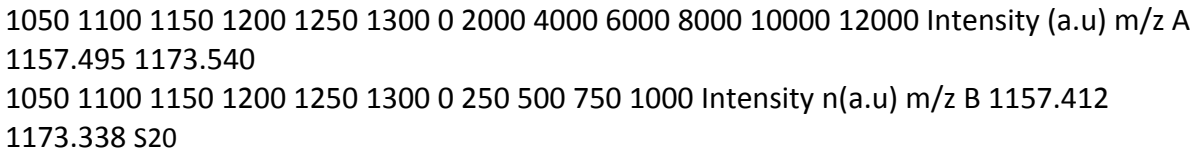
548 µm

(c)

548 µm

Figure S7. Sample morphology. Analyte: neocarratetraose 4_1,4_3-disulfate disodium salt (NCT), matrix: (a) *E*-SA (b) Z-SA and (c) I-*E*-SA. Circular spot surface, 9.62 mm^2. Digital images from Bruker Ultraflex II TOF/TOF. S18

Figure S8. Effect of the irradiation time of the *E*-SA methanolic solution on the LDI mass spectrum of *E*-SA. Negative ion mode, detected as [M-H]-. (A) *Z*-SA; (B) *E*-SA; (C) I-*E*-SA 3h and (D) I-*E*-SA 8h. S19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1157.495 1173.540
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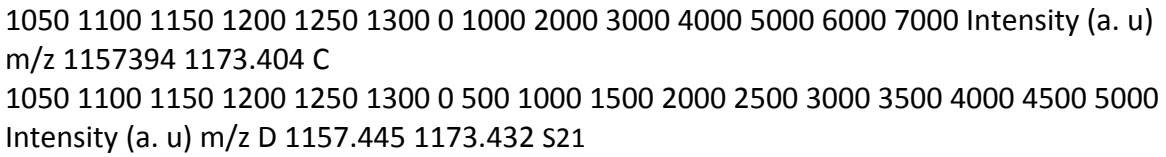
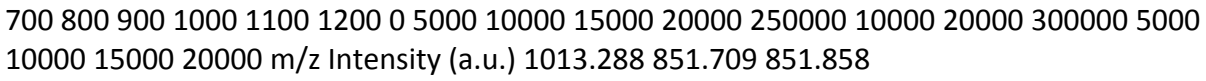
1173.338


1050 1100 1150 1200 1250 1300 0 1000 2000 3000 4000 5000 6000 7000 Intensity (a. u)
m/z 1157394 1173.404 C

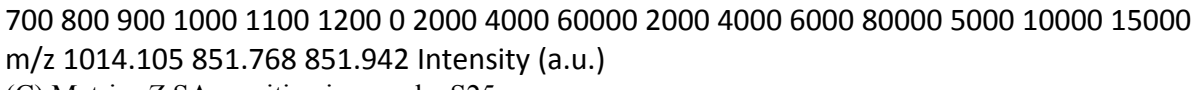
1050 1100 1150 1200 1250 1300 0 500 1000 1500 2000 2500 3000 3500 4000 4500 5000
Intensity (a. u) m/z D 1157.445 1173.432
S21

Figure S9. Effect of of irradiation of the *E*-SA methanolic solution on the performance of *E*-SA as matrix. Positive ion mode. Analyte b-CD (m.w. 1134.98) detected as $[M+Na]_+$ and $[M+K]_+$. Matrix: (A) *Z*-SA; (B) *E*-SA; (C) pre-prepared *Z*-SA+*E*-SA 1:1 (mol/mol); (D) irradiated *E*-SA solution (I-*E*-SA) 3h; (E) irradiated *E*-SA solution (I-*E*-SA) 8h. S22

700 800 900 1000 1100 1200 0 1000 2000 3000 4000 50000 2000 4000 6000 80000 5000 10000 15000 20000 25000 m/z1013.898 852.251 Intensity (a.u.) 852.557
(A) Matrix: nHo, positive ion mode.



(B) Matrix: DHBA, positive ion mode.

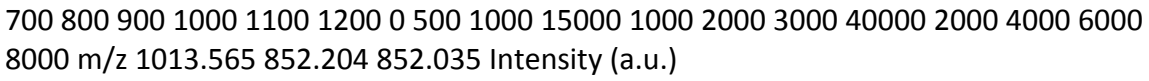


(C) Matrix: *Z*-SA, positive ion mode. S25

(D) Matrix*: E*-SA, positive ion mode.

700 800 900 1000 1100 1200 0 500 1000 15000 500 1000 1500 20000 2000 4000 6000 8000 m/z 1013.947 851.795 Intensity (a.u) 851.799

(E) Matrix: I-*E*-SA 30 min., positive ion mode. S27



(F) Matrix: I-*E*-SA 1h, positive ion mode.

700 800 900 1000 1100 1200 0 500 1000 1500 20000 1000 2000 3000 40000 5000 10000 15000 m/z1013.408 Intensity (a.u.) 851.860 851.997
(G) Matrix: I-*E*-SA 2 h, positive ion mode.

Figure S10. Effect of the irradiation time of the *E*-SA methanolic solution on the performance of *E*-SA as matrix. Positive ion mode. Analytes: F5 (m.w. 828.71), M5 (m.w.828.71) and M6 (m.w. 990.86), detected as $[M+Na]^+$ and $[M+K]^+$. Matrix: (A) nHo; (B) DHBA; (C) *Z*-SA; (D) *E*-SA; (E) I-*E*-SA 30min; (F) I-*E*-SA 1h and (F) I-*E*-SA 2h.