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Evaporative derivatization of phenols with 2-sulfobenzoic anhydride for detection by matrix-assisted laser desorption/ionization mass spectrometry

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Yuanyuan Yao, Poguang Wang and Roger Giese*

Department of Pharmaceutical Sciences and Barnett Institute, Bouve College, Northeastern University, Boston, MA 02115, USA

RATIONALE: Phenols are an important class of analytes, for example as bioactive environmental contaminants. Towards a goal of improving their detection by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) or MALDI-tandem time-of-flight (TOF/TOF)-MS, we studied their derivatization with 2-sulfobenzoic anhydride (SBA). We chose SBA for this purpose since it is commercially available, inexpensive, and forms an anionic derivative.

METHODS: Under the selected conditions developed here for phenols, a reaction mixture of one or more of such compounds in acetonitrile containing SBA and 4-dimethylaminopyridine (DMAP) is evaporated to a solid, heated at 60 °C for 1 h, redissolved in 50% acetonitrile containing matrix, spotted onto a MALDI target, and subjected to negative ion MALDI-TOF/TOF-MS.

RESULTS: While conventional (solution-phase) reaction of 4-phenylphenol (model analyte) with SBA and DMAP only gave a 47% yield of SBA-tagged 4-phenylphenol, evaporative derivatization as above gave a 96% yield, and 25 pmol (4.3 ng) of 4-phenylphenol could be detected in this way by MALDI-TOF/TOF-MS at signal-to-noise ratio (S/N) = 260, whereas even 1 nmol of the nonderivatized phenol was not detected in the absence of derivatization. A wide range of responses was observed when a mixture of 15 phenols was derivatized, with the higher responses coming from phenols with a pK_a value above 9. Without derivatization, phenols with pK_a values below 5 were the most readily detected.

CONCLUSIONS: Evaporative derivatization with SBA (a convenient reagent) can improve the detection of phenols with relatively high pK_a values (above 9) by negative ion MALDI-TOF-MS, and accomplish this in the absence of post-derivatization reaction cleanup. Copyright © 2014 John Wiley & Sons, Ltd.

As reviewed by Zaikin and Halket,^[1] ionic tagging of small molecules to increase their response by soft ionization techniques such as electrospray ionization (ESI) and matrixassisted laser desorption/ionization (MALDI) is of increasing interest. Other benefits can accrue such as increased selectivity. One of the reagents which has been studied in this way is 2-sulfobenzoic anhydride (SBA). While the compound is commercially available and relatively inexpensive, it has been explored to a limited degree in terms of the number of publications reporting its analytical use; how much it enhances analyte response; and its performance when applied to a small amount of analyte.

In more detail, the analytical studies of SBA to date are as follows. Peptides were tagged with SBA to improve their fragmentation (for sequencing purposes) in the post-source decay positive ion mode of MALDI.^[2,3] SBA has been used to derivatize a diversity of alcohols (including phenol, glucose, and mandelic acid) for detection by negative

ion MALDI-time-of-flight mass spectrometry (TOF-MS).^[4] Multiple products were reported from multi-hydroxy compounds, whose formation was attributed to steric effects. The amount of each alcohol reacted (calculated per hydroxy function) was 0.1 mmol. Yields were not defined. The limit of detection (LOD) was reported as 2.5 pmol for SBA-tagged methanol, based on testing a diluted reaction mixture, assuming a reaction yield of 100%; apparently this value was obtained by extrapolating from a measurement at a higher concentration. It was not clear whether a blank sample was derivatized to rule out contamination. Zu and coworkers derivatized fatty alcohol ethoxylate non-ionic surfactants at the 0.05 mmol level using SBA in the presence of 4dimethylaminopyridine (DMAP), followed by diluting the reaction mixture and subjecting it to liquid chromatography (LC)/ESI-MS or flow injection ESI-MS.^[5] The yield was reported to be about 95% based on the assumption that the product (SBA-tagged alcohol) and starting alcohol gave the same response by ESI-MS. SBA has also been used to modify proteins^[6,7] and industrial polymers.^[8]

Towards a goal of improving the detection of phenols by MALDI-MS, we have extended the work of others concerning derivatization with SBA, and this manuscript reports our progress. The detection of phenols is of broad interest mainly because some are bioactive as biomolecules or xenobiotics.

^{*} Correspondence to: R. Giese, Department of Pharmaceutical Sciences and Barnett Institute, Bouve College, Northeastern University, 206 The Fenway, 360 Huntington Ave., Boston, MA 02115, USA. E-mail: r.giese@neu.edu



Mass spectrometry is used commonly to detect phenols in both of these roles, usually as part of a gas chromatography $(GC)/MS^{[9-12]}$ or $LC/MS^{[13-16]}$ system.

EXPERIMENTAL

Materials

4-Phenylphenol (4PP), 4-nonylphenol, bisphenol A (BPA), 2-sulfobenzoic anhydride (SBA), 4-dimethylaminopyridine (DMAP), acetonitrile (ACN, anhydrous, 99.8%), and α -cyano-4-hydroxycinnamic acid (CCA) were from Sigma-Aldrich (St. Louis, MO, USA). CCA matrix was 5 mg/mL of CCA in ACN/H₂O (1:1, v/v). Phenol Calibration Mix (12 components Cat. No. 31694) was purchased from Restek (Bellefonte, PA, USA). Water (distilled, HPLC grade) and ACN (HPLC grade) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Glass vials with inserts (product No. 09-1200-101) and screw thread caps (product No. 09-0040R) were purchased from Microliter Analytical Supplies (Suwanee, GA, USA).

Non-evaporative derivatization of 4-phenylphenol with SBA

The conditions of Zu and coworkers^[5] for derivatization of polyols with SBA were employed. Briefly, 4PP (8.5 mg, 0.05 mmol), SBA (92 mg, 0.5 mmol), and DMAP (0.1 mg, 8.2×10^{-4} mmol, 1% w/w of 4PP) were dissolved in 1 mL of ACN. After sitting at room temperature for 1 h, the mixture was kept at 80 °C for 2 h. The reaction was also conducted at room temperature for 24 h. After the reaction, the mixture was diluted 100-fold with ACN/H₂O (20:80, v/v) for HPLC analysis.

Evaporative derivatization of 4-phenylphenol with SBA

4PP was used as a model phenol to optimize the conditions for evaporative derivatization. For the initial studies, one equivalent of 4PP and ten equivalents of SBA were combined in 2% (w/w of 4PP) of DMAP in 1 mL ACN to reach a concentration of 4PP (5×10^{-2} M, 5×10^{-3} M, and 5×10^{-4} M). A 50 µL aliquot was pipetted into a glass vial with an insert and evaporated in a Speed Vac (Savant Instruments, Saroor Nagar, Hyderbad, India; the manufacture-specified vacuum was 0.6 Torr) at room temperature for 40 min. The resulting solid was incubated at 60 °C in a sand bath (Reacti-therm, Pierce, Rockford, IL, USA) for 1 h with the bottom of the cap 4 mm above the sand. For subsequent HPLC-UV analysis after derivatization, each reaction mixture was dissolved in 50 µL of 50% ACN. The reaction mixtures with initial 4PP concentrations of 5×10^{-2} M, 5×10^{-3} M, and 5×10^{-4} M were diluted, respectively, 100-fold, 10-fold, and 10-fold with ACN/H₂O (20:80, v/v) prior to HPLC analysis. In addition, 5 μL of a reaction mixture having 5×10^{-6} M 4PP along with 5×10^{-3} M SBA and 1.6×10^{-7} M DMAP was subjected to evaporative derivatization prior to addition of 10 µL of CCA matrix and deposition of 1 µL onto the target plate for analysis by MALDI-MS.

Evaporative derivatization of 15 phenols with SBA

A mixture of 15 phenols (4PP, 4-nonylphenol, BPA, and a 12-component Phenols Calibration Mix) in 1 mL of ACN was set up as follows: 5×10^{-4} M each component phenol, 5×10^{-3} M SBA, and 2% (w/w of total phenols) DMAP. A 50 μ L aliquot was evaporated and heated at 60 °C for 1 h followed by dissolving in 50 μ L of ACN /H₂O (1:1), and 1 μ L was then combined with 10 μ L of CCA for subsequent analysis of 1 μ L by MALDI-MS.

Analysis by HPLC-UV

A 10 μ L aliquot of the diluted sample after derivatization was injected into a Hewlett Packard Series 1100 HPLC system. The HPLC column was an AQUASIL (Thermo Fisher Scientific) C18 column (150 × 2.1 mm, 5 μ m) that was kept at room temperature and operated at a flow rate of 0.21 mL/min. Before each run, the column was equilibrated for 10 min with 2:98, v/v, ACN/H₂O. The following solvent gradient was applied: 2% ACN for 3 min, ramp to 50% ACN over 13 min, hold for 4 min, ramp to 100% ACN over 5 min, and hold for 5 min. Absorbance detection was at 256 nm.

Analysis by MALDI-MS (MALDI-TOF-MS or MALDI-TOF/TOF-MS)

MALDI-MS analysis was performed on a model 5800 MALDI-TOF/TOF-MS instrument (AB SCIEX, Framingham, MA, USA) in negative ion, reflectron mode with a delay time of 100 ns, with 1200 laser pulses being averaged to generate a spectrum. MS/MS was performed by TOF/TOF with the metastable-suppressor on and the collision-induced dissociation (CID) off. A mass resolution window of 400 (instrumental setting) was used. This setting (viz. 400) combined with the precursor ion mass defines the mass window for MS² (done automatically by the software). The relative laser intensity was set at ~2500 for MS mode and ~ 3500 for MS/MS mode. For the direct analysis of the derivatized phenol mixture, a 1 µL aliquot of the redissolved reaction mixture in ACN/H₂O (1:1, v/v) was mixed with 10 µL CCA matrix, and 1 µL was deposited onto the MALDI plate. For the analysis of the collected HPLC peak fraction for SB-4PP after the derivatization reaction, 10 µL CCA matrix was added to the dried fraction, and 1 µL was deposited onto the MALDI plate.

Evaporative derivatization of pentachlorophenol with SBA

This reaction was conducted on 50 μ L of 0.05 M pentachlorophenol in the same way as the corresponding sample of 4PP described above, except that, in addition to a reaction time of 1 h at 60 °C, reaction temperatures of 80 °C and 100 °C were studied. This was followed by HPLC-UV analysis and, in turn, peak collection for analysis by MALDI-TOF, as above for 4PP.

RESULTS AND DISCUSSION

To optimize the derivatization of phenols by SBA, we initially tested 4-phenylphenol (4PP) as a model analyte, and relied on HPLC-UV and/or MALDI-MS to assess the product mixture. A scheme for the derivatization and analysis of this (and later other phenols) by MALDI-MS is shown in Fig. 1. At first we studied the two kinds of reaction conditions reported by Zu and coworkers.^[5] Since both the 4PP and the desired product, SBA-tagged 4-phenylphenol (SB-4PP), have relatively simple structures, and the HPLC chromatograms displayed no significant side products, we relied on the disappearance of the peak for 4PP to estimate the yield. (This way of assessing the yield is validated further below.) By this strategy, the yield of SB-4PP was about 52% (n = 3, coefficient of variance (CV) = 6%) when the reaction was conducted by keeping it at room temperature for 1 h prior to heating it at 80 °C (one type of condition employed previously^[5]). A representative HPLC chromatogram from this experiment is shown in Fig. 2 (A), along with that from an unheated reaction at time zero (inset of Fig. 2(A)). Peak 1 was confirmed to be SB-4PP by collecting it and subjecting it to MALDI-TOF-MS and MALDI-TOF/TOF-MS analysis (see below). Conducting the reaction at room temperature for 24 h (second type of condition reported previously^[5]) gave a yield of 3% (n=3, CV = 0.17%; data not shown).

We next explored 'evaporative derivatization conditions' to potentially improve the yield (by increasing the concentration of the reagent, and perhaps also by creating a different reaction microenvironment), and also potentially to facilitate or even avoid post-derivatization sample cleanup (by minimizing the amounts of the derivatization reagents) when 4PP is reacted with SBA. The reaction mixture was evaporated prior to incubating it at room temperature or at an elevated temperature. We initially tested the following concentrations of reactants in ACN (same as those tested above) prior to evaporation: 5×10^{-2} M 4PP, 5×10^{-1} M SBA, and 1.6×10^{-3} M DMAP. Evaporation of a 50 µL aliquot of a 1 mL reaction solution was carried out at room temperature for 40 min at 0.6 Torr, yielding a solid. Keeping this solid at room temperature for 3 days gave a 48% yield of SB-4PP based on HPLC-UV (the yield progressively climbed to this level during this period of time; data not shown). A longer reaction time at room temperature was not studied.

The evaporative derivatization reaction was faster and the yield was higher at an elevated temperature. After studying various times and temperatures (see below), we selected 1 h at 60 °C, which gave a yield of 96% (n=3, CV=1%), since the other conditions tried (longer times and higher temperatures) gave little improvement in yield, and a higher temperature or longer time risks an increase in noise. A representative HPLC chromatogram is shown in Fig. 2(B),



Figure 1. Scheme for detection of phenols by derivatization with 2-sulfobenzoic anhydride (SBA) followed by mass spectrometry, where MS designates MALDI-TOF-MS, and MS/MS designates MALDI-TOF/TOF-MS, each in negative ion mode.



Figure 2. HPLC chromatograms of reaction mixtures of 4PP, SBA and DMAP: (A) non-evaporative derivatization where the reaction mixture was heated at 80 °C for 2 h and (B) evaporative derivatization where the reaction mixture was evaporated and then heated at 60 °C for 1 h. Peak 1: SB-4PP; peak 2: 4PP. For HPLC conditions, see Experimental section. Însets: unheated reactions at time zero.

along with that from an unheated reaction at time zero in the inset. The early-eluting peak in Fig. 2(B) was 10% higher when the phenol was not present in the reaction mixture (unreacted or hydrolyzed reagents contributed to this peak, so the peak was slightly increased in the blank since some of these reagents had reacted with the phenol). This further validated our way of assessing the yield. Examples of results from other conditions are as follows (expressed as mean ± CV) where the reaction time was 2 h and n = 3 in each case: $59 \pm 1\%$ at 40 °C, $97 \pm 0.2\%$ at 60 °C, and $98 \pm 0.3\%$ at 80 °C (data not shown). Direct analysis of the evaporative reaction mixture (after addition of CCA matrix), using the condition 60 °C for 1 h, by MALDI-TOF-MS, gave the data shown in Fig. 3(A), with a peak for deprotonated SB-4PP at m/z353.050 (exact mass 353.048), and the data from a blank reaction (no 4PP) in Fig. 3(B). (The isotope peak at m/z354.050 in Fig. 3(A) falsely appears to be too high since the corresponding nonisotopic peak is saturated.) Corresponding data recorded by MALDI-TOF/TOF-MS are shown in Fig. 4



Figure 3. Negative ion MALDI-TOF-MS spectra: (A) reaction mixture of 4PP and SBA after 1 μ L was combined with 10 μ L CCA matrix and spotted onto the target for MALDI-TOF-MS giving a peak for deprotonated SB-4PP at *m*/z 353.050 (exact mass 353.048); the reaction was conducted by non-evaporative derivatization at 60 °C for 1 h with the initial reaction mixture of 5 × 10⁻² M: 4PP, 5 × 10⁻¹ M SBA, and 1.6 × 10⁻³ M DMAP. (B) 1 μ L of ACN combined with CCA matrix, showing an absence of this peak.



Figure 4. Negative ion MALDI-TOF/TOF-MS spectra of: (A) same sample as in Fig. 3(A) and (B) same sample as in Fig. 3(B), after selection of the deprotonated SB-4PP precursor ion at m/z 353.

(A) (showing a peak for the 4-phenylphenolate product ion at m/z 169 derived from the deprotonated molecule at m/z 353) and in Fig. 4(B) (blank: no 4PP).

The reaction of 4PP with SBA was next studied at lower concentrations of 4PP under the selected reaction conditions (60 °C, 1 h), while also lowering the concentration of SBA and DMAP, but keeping the relative molar amounts of 4PP, SBA, and DMAP constant (1:10:0.03). We observed by

HPLC-UV that the yield of SB-4PP was unchanged even when the following concentrations were set up prior to evaporation: 5×10^{-4} M 4PP, 5×10^{-3} M SBA, and 1.6×10^{-5} M DMAP. Lower concentrations (with the usual dilution after the reaction) than this of 4PP could not be detected reliably by HPLC-UV. However, SB-4PP product (at *m*/*z* 353.050) could be detected by both MALDI-TOF-MS (Fig. 5(A)) and MALDI-TOF/TOF-MS (peak at *m*/*z* 169 in Fig. 5(B)), even when the





Figure 5. (A) Negative ion MALDI-TOF-MS spectrum after evaporative derivatization at 60 °C for 1 h with the initial reaction mixture of 50 μ L 5 × 10⁻⁶ M 4PP, 5 × 10⁻³ M SBA, and 1.6 × 10⁻⁷ M DMAP, showing a peak for deprotonated SB-4PP at *m*/z 353.050. (B) Negative ion MALDI-TOF/TOF-MS spectrum of the same sample after selection of the ion at *m*/z 353 for deprotonated SB-4PP, showing a peak at *m*/z 169 for the phenolate product ion of SB-4PP (see Fig. 1). Inset of (B): Spectrum **a**: negative ion MALDI-TOF/ TOF-MS spectrum after evaporative derivatization 60 °C for 1 h of an initial reaction mixture of 5 μ L 5 × 10⁻⁶ M 4PP, 5 × 10⁻³ M SBA, and 1.6 × 10⁻⁷ M DMAP; the peak at *m*/z 169 has a S/N of 260. Spectrum **b**: blank reaction (no 4PP).

following initial solution concentrations were tested: 5×10^{-6} M 4-PP, 5×10^{-3} M SBA, and 1.6×10^{-7} M DMAP. Since the volume of this reaction solution prior to evaporation was 50 µL, about 43 ng of 4PP was detected in this way. While evaporating 5 µL of this reaction solution (containing 4.3 ng or 25 pmol) did not yield a peak for the product by MALDI-TOF-MS, detection by MALDI-TOF/TOF-MS was successful (S/N = 260 for *m*/*z* 169), as seen in spectrum **a** in the inset of Fig. 5(B), where the spectrum from the blank reaction (no 4PP) is also shown (spectrum **b** in the inset).

Evaporative derivatization of a mixture of phenols

A mixture of 15 phenols (structures shown in Table 1) was reacted with SBA under evaporative derivatization conditions, where the concentration of each phenol was 5×10^{-4} M in the 50 µL solution prior to evaporation, and

the corresponding concentrations of SBA and DMAP were 5×10^{-3} M and 1.6×10^{-5} M, respectively. The relative responses (relative to 4-nonylphenol; see below) of the products were obtained by directly subjecting an aliquot of the reaction mixture, after matrix addition, to MALDI-TOF-MS and MALDI-TOF/TOF-MS, giving the data shown in Fig. 6. This data is tabulated in Table 1, along with the pK_a values of the phenols. Note that the values for relative response in the table reflect the combined variation in both reaction yield and S/N by the two MALDI techniques. The response of 4-nonylphenol was arbitrarily assigned a value of 100 independently for both the TOF and the TOF/TOF data since it gave the highest S/N in each case. The nature of the cited ions is defined generically in Fig. 1. Overall, it is clear that phenols can differ widely in their response by the method presented here.



Number	Structure	Deprotonated molecule by TOF (m/z)	R-Phenolate by TOF/TOF (<i>m/z</i>)	pK _a
1	но	100 (403.158)	100 (219.17)	10.4
2	ОН	29.5 (353.048)	16.5 (169.07)	9.6
3	но	25.0 (305.048)	4.0 (121.07)	10.6
4	HO	12.3 (324.994)	3.8 (141.01)	9.6
5	HO	11.6 (411.090)	7.1 (227.11)	9.6, 11.3
6	но	8.6 (291.033)	1.1 (107.05)	10.3
7	но	6.4 (277.017)	1.0 (93.03)	10.0
8	ноа	0.3 (344.939)	0.1 (160.96)	7.9
9 and 10	O 2 N OH OH NO2	0.1 (322.002)	0.2 (138.02)	7.2
11	ю ⁰ ; ^N	N/D (381.003)	N/D (197.02)	4.3
12	0 2 N H0	N/D (366.987)	N/D (183.00)	4.1
13	ю — С	N/D (310.978)	N/D (127.00)	8.6
				(Continues)

Table 1. Relative yield/response values by MALDI-MS (data from Fig. 6) for phenols after evaporative derivatization with SBA^a





^aNames of compounds: 1) 4-nonylphenol; 2) 4-phenylphenol (4PP); 3) 2,4-dimethylphenol; 4) 4-chloro-3-methylphenol; 5) bisphenol A (BPA); 6) o-cresol; 7) phenol; 8) 2,4-dichlorophenol; 9) 2-nitrophenol; 10) 4-nitrophenol; 11) 2-methyl-4,6-dinitrophenol; 12) 2,4-dinitrophenol; 13) 2-chlorophenol; 14) 2,4,6-trichlorophenol; 15) pentachlorophenol. ^bND: not detected or not readily detected.



Figure 6. Negative ion MALDI-TOF-MS spectra of: (A) reaction mixture of 15 phenols (see Table 1) in which evaporative derivatization with SBA (5×10^{-3} M) and DMAP (2% w/w of total phenols) was performed at 60 °C for 1 h, where each phenol was 5×10^{-4} M and (B) reaction blank (phenols not present).

There could be several reasons that eight of the phenols (8–15) gave little or no response by MALDI-TOF-MS after derivatization with SBA, such as incomplete derivatization, instability of the derivatives in the MALDI step, and variation in the efficiency of desorption. It is interesting that all these compounds have relatively low pK_a values (below 9). We tested pentachlorophenol with reaction monitoring by HPLC-UV, and HPLC-peak collection-MALDI-MS, to gain some insight. The yield of SBA-tagged pentachlorophenol

was about 1% at 60 °C, about 2% at 80 °C, and about 25% at 100 °C after 2 h of an evaporative derivatization reaction, based on disappearance of the peak for starting material. Longer reaction times increased the formation of side products without significantly increasing the yield. The HPLC chromatogram from the latter reaction (100 °C, 2 h) is shown in Fig. 7. The new peak (peak 1) was confirmed to be SBA-tagged pentachlorophenol by collection and analysis by MALDI-TOF-MS in positive ion mode, giving



Figure 7. HPLC chromatogram of the reaction mixture of pentachlorophenol, SBA and DMAP after evaporative derivatization at 100 °C for 1 h. Peak 1: SBA-derivatized pentachlorophenol. Peak 2: underivatized pentachlorophenol. For concentrations and HPLC conditions, see Experimental section.

an ion for $[M-H+2Na]^+$ at m/z 492.810 (exact mass 492.801; data not shown). The observed isotope pattern of this deprotonated disodiated molecule matched the calculated isotope pattern (data not shown).

In negative ion mode, no deprotonated molecule was observed from the collected HPLC peak (peak 1 in Fig. 7) from the above reaction of pentachlorophenol; instead peaks for [C₆Cl₅O]⁻ (*m*/*z* 264.839), [C₆Cl₅O+CCA-H+Na]⁻ (475.853), and $[C_6Cl_5O+C_6Cl_5O+Na]^-$ (552.651) were seen (data not shown); peak ratio 7:6:2. This prompted us to re-evaluate the MALDI-TOF-MS data related to pentachlorophenol from the reaction mixture of 15 phenols. Indeed, peaks for [C₆Cl₅O]⁻ and [C₆Cl₅O+CCA-H+Na]⁻ were seen. For pentachlorophenol, one can speculate that the failure to readily detect the derivatized compound in the reaction of 15 phenols, according to the strategy summarized in Table 1, was mainly due to a combination of low reaction yield (apparently from steric and/or inductive effects), spread of the signal in MALDI-TOF-MS over several ions including isotope peaks, and pronounced instability of the deprotonated molecule in the ion source. To different degrees, these same factors may contribute to the behavior of the other phenols in Table 1 that gave little or no response under the conditions employed. Overall, it is seen that the phenols with relatively high pK_a values (above 9, compounds 1-7) are much more detectable (>10-fold) than those with pK_a values below 9 (compounds 8–15). The highest responders are monophenols with a higher degree of alkyl or aryl bulk, and a p K_a above 9 (compounds 1–3).

We also tested the nonderivatized phenols by negative ion MALDI-MS. The amount of each compound applied to the target was 1 nmol irrespective of whether it was tested individually (compounds 1, 2, 5 and 15) or as a mixture (compounds 3, 4, and 6–15). At a laser setting of 3800, the compounds either gave no response (compounds 1, 2, 5 and 6); encountered matrix interference (3 and 7); gave a weak signal (S/N = 77 for 4, 43 for 8, 47 for 13, and 121 for 14); gave a strong signal (compounds 9 and 10, S/N = 1100); or gave a saturated (off-scale) signal (11, 12 and 15). The highest laser setting (2400) that kept the peak on scale for compounds 1, 8, 9, 10 and 14, while the peaks for compounds 11 and 12 were

still off scale. Reducing the laser setting to 2200 brought the peaks for **11** and **12** on scale (S/N = 1860 and 1760, respectively) and lowered the S/N of compound **15** to 219. We note that the highest responders (**11**, **12** and **15**) have the lowest pK_a values (4.1–4.7). Overall, derivatization with SBA therefore is somewhat complementary to detection by negative ion MALDI-MS without derivatization, since phenols giving little or no response when underivatized (compounds **1**, **2**, **4**, **5**, **6**) tended to be readily or highly detected after derivatization (compounds **1**–7).

CONCLUSIONS

2-Sulfobenzoic anhydride has some properties, either wellknown or introduced here, that encourage its further study as an analytical derivatization reagent for phenols with a relatively high pK_a (above 9) to improve their detection by negative ion MALDI-MS: commercial availability at low cost; high reaction yield by evaporative derivatization; and effectiveness under this condition without post-derivatization sample cleanup. In the absence of derivatization, phenols with pK_a values below 5 were the most readily detected.

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