Derivatization of bisphenol A and its analogues with pyridine-3-sulfonyl chloride: multivariate optimization and fragmentation patterns by liquid chromatography/Orbitrap mass spectrometry

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RATIONAL E: Due to the growing restrictions on the use of bisphenol A (BPA), several other bisphenols are gaining importance as substitutes for BPA in a variety of applications. There is, therefore, a real need for selective and sensitive methods based on mass spectrometry which will allow the human exposure to these new bisphenols to be assessed.

METHODS: Derivatization of BPA and its substitutes with pyridine-3-sulfonyl chloride is used to enhance the detection capability of bisphenols by electrospray ionization mass spectrometry. A multivariate experimental design, Box-Behnken response surface, was used to evaluate the influence of the main variables potentially affecting the derivatization yield. Fragmentation patterns for all the derivatized bisphenols were acquired by high-resolution/accurate-mass Orbitrap mass spectrometry.

RESULTS: Temperature and pH were identified as the most important factors affecting the derivatization yield of bisphenols. Fragmentation of the protonated molecules produced abundant analyte-specific product ions. Most of the derivatized bisphenols showed significant improvements in their signal-to-noise ratios compared with the underivatized forms. The stability of these derivatives was demonstrated through several freeze/thaw cycles, short-term room temperature and long-term cold storage.

CONCLUSIONS: Derivatization of BPA and its structural analogues with pyridine-3-sulfonyl chloride is proposed as a specific, sensitive, high-throughput approach to their analysis by liquid chromatography coupled to electrospray ionization mass spectrometry. © 2015 The Authors. Rapid Communications in Mass Spectrometry Published by John Wiley & Sons Ltd.

Because of the extensive use of bisphenol A (BPA) in the manufacture of many consumer products, human exposure to this chemical has become widespread. BPA is mainly used in the production of polycarbonate plastics and epoxy resins, but also in other polymers and resins (such as polysulfone, polyester and vinyl ester resins), in the synthesis of certain flame retardants, and as a developer in thermal paper.

These BPA-containing materials are employed in a wide variety of applications, including food and liquid containers, cookware, toys, inner linings of metal cans and bottle tops, surface coatings, medical devices, dental fillings and cash register receipts.

BPA is known to have estrogenic properties and to cause adverse health effects in animals. Its effects on mammary glands, on brain and behavioral development, as well as on metabolism and obesity, have been reported. Nevertheless, clear epidemiological evidence of any health effects in humans is still missing. Very recently, the European Food Safety Authority (EFSA) conducted a thorough review of the risks to public health related to the presence of BPA in foodstuffs. According to EFSA, BPA poses no health risk to consumers because current exposure to the chemical is too low to cause harm. However, the EFSA’s Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) has recommended that the Tolerable Daily Intake (TDI) for BPA should be lowered on a temporary basis from 50 μg/kg bw/day to 4 μg/kg bw/day.

In the European Union (EU), BPA is permitted for use in food contact materials under Commission Regulation (EU) No 10/2011, relating to plastic materials and articles intended to come into contact with foodstuffs. Nevertheless, the European Commission has recently banned the use of BPA, as a precaution, for the manufacture of polycarbonate infant feeding bottles. These restrictions together with the growing public concern about its toxicity have forced the plastic industry to look for alternatives to BPA. Most of them consist of compounds that are very similar in structure to BPA, belonging to the same chemical group of p,p'-bisphenols (Table 1). Among these structural analogues, bisphenol S (BPS), bisphenol F (BPF), bisphenol B (BPB) and bisphenol AF (BPAF) have arisen in recent years as the main BPA replacements in certain products. Limited studies have
shown that BPA analogues possess toxicities (e.g., acute toxicity, genotoxicity, and estrogenic activity) similar to BPA.\cite{2,9,10} For instance, Grignard et al.\cite{10} reported comparable weak estrogenic transcriptional activities for BPS and BPA.

BPS, BPF, and other BP analogues such as bisphenol B (BPB) have been identified as emerging contaminants in beverages and foodstuffs,\cite{11-13} environmental matrices,\cite{14,15} and biological samples.\cite{16} Nevertheless, data on the occurrence of bisphenol analogues are still scarce, mainly due to the lack of sensitive methods for their determination at trace levels.

Due to its selectivity and sensitivity, liquid chromatography coupled to tandem mass spectrometry (LC/MS/MS) equipped with an electrospray ionization (ESI) source has become one of the most prominent analytical techniques.\cite{17} According to their pKa values (Table 1), bisphenols are neutral compounds under the acidic to neutral pH ranges generally necessary for robust LC separations; therefore, their efficiency in ESI is in general rather low. Chemical derivatization of poorly ionizable analytes is often used to enhance the detection capability in ESI-MS.\cite{17,18} In this context, derivatization of BPA using dansyl chloride has become a quite common procedure to increase its ionization efficiency. Thus, several authors

| Compound       | Acronym | CAS number | Monoisotopic mass (u) | Log  $K_{ow}$ $^{a}$ | pKa $^{a}$ | Structure |
|----------------|---------|------------|-----------------------|----------------------|-----------|-----------|
| Bisphenol S    | BPS     | 80-09-1    | 250.0300              | 2.32                 | 7.42-8.03 | ![Structure Image](https://example.com/structure_image.png) |
| 4,4'-Bisphenol F | BPF     | 620-92-8   | 200.0837              | 3.46                 | 9.84-10.45| ![Structure Image](https://example.com/structure_image.png) |
| Bisphenol E    | BPE     | 2081-08-5  | 214.0994              | 3.74                 | 9.81-10.42| ![Structure Image](https://example.com/structure_image.png) |
| Bisphenol A    | BPA     | 80-05-7    | 228.1150              | 4.04                 | 9.78-10.39| ![Structure Image](https://example.com/structure_image.png) |
| Bisphenol B    | BPB     | 77-40-7    | 242.1307              | 4.49                 | 9.77-10.38| ![Structure Image](https://example.com/structure_image.png) |
| Bisphenol Z    | BPZ     | 843-55-0   | 268.1463              | 4.91                 | 9.76-10.37| ![Structure Image](https://example.com/structure_image.png) |
| Bisphenol AP   | BPAP    | 1571-75-1  | 290.1307              | 5.18                 | 9.66-10.27| ![Structure Image](https://example.com/structure_image.png) |
| Bisphenol AF   | BPAF    | 1478-61-1  | 336.0585              | 4.77                 | 9.13-9.74 | ![Structure Image](https://example.com/structure_image.png) |
| Bisphenol P    | BPP     | 2167-51-3  | 346.1933              | 6.72                 | 9.78-10.38| ![Structure Image](https://example.com/structure_image.png) |

\(^{a}\text{Chemicalize.org by ChemAxon.}\)\cite{41}
have applied derivatization of BPA with dansyl chloride to increase its ionization efficiency in different biological,\textsuperscript{19–22} environmental and food samples.\textsuperscript{20} Despite the great improvement in ESI+ ionization of the resulting BPA derivative, the MS/MS fragmentation of the resulting ion yields only reagent-specific product ions, which drastically reduces the specificity of the method. Chemical derivatization using pyridine-3-sulfonyl (PS) chloride has been very recently applied for the determination of BPA in different kinds of biological matrices with successful results with regard to both sensitivity and specificity.\textsuperscript{[23–26]} This derivatization reagent has been reported to present a major advantage over the widely used dansyl chloride, since MS/MS transitions involve analyte-specific product ions rather than reagent-specific product ions.\textsuperscript{[23,24,27]} In this way, interferences arising from matrix components, which are of special concern when analyzing complex samples, are reduced.

Although all the studied bisphenols belong to the same chemical group as BPA, structural differences among them may significantly affect their reactivity with PS, which makes an individualized study of their reaction with this novel derivatization reagent necessary. For instance, the dimethylmethylene group of BPA is replaced in BPS with a sulfone group, which definitively results in a significantly higher polarity and acidity (Table 1).

Thus, the aim of the present work was to evaluate PS chloride as a derivatization reagent not only for BPA, but also for its main analogues – BPS, BPF, bisphenol E (BPE), BPP, bisphenol Z (BPZ), BPAF, bisphenol AP (BAP) and bisphenol P (BFP) – in order to improve the detection capabilities of these compounds by LC/ESI-MS. To the best of our knowledge, this paper describes for the first time the use of this reagent for the derivatization of bisphenols other than BPA. A multivariate experimental design, Box–Behnken response surface, was used to assess the influence of the main variables potentially affecting the derivatization yield as well as to obtain the optimal conditions.

In order to demonstrate the analyte-specificity of the product ions, the MS/MS fragmentation patterns of the [M +H]\textsuperscript{+} ions of the resulting PS derivatives were examined using a high-resolution/accurate-mass hybrid ion trap-Orbitrap mass spectrometer. Accurate-mass information allowed the identity of the product ions to be confirmed with high confidence. This information will permit a more rational selection of structurally specific product ions for multiple reaction monitoring (MRM) methods in tandem mass spectrometry instruments, minimizing the possibility of reporting false positives or false negatives.

**EXPERIMENTAL**

**Chemicals and materials**

Bisphenol A (≥99%), bisphenol AF (≥97%), bisphenol AP (≥99%), 4,4′-bisphenol F (≥98%), bisphenol P (≥99%), bisphenol S (≥98%), bisphenol Z (≥98%) and the derivatization reagent, pyridine-3-sulfonyl chloride hydrochloride (≥98%), were purchased from Sigma-Aldrich (Diegem, Belgium). Bisphenol B (≥98%) and bisphenol E (≥98%) were obtained from TCI (Zwijndrecht, Belgium). Chemical structures, octanol-water partition coefficients (log K\textsubscript{ow}) and pKa values of the analyzed compounds are shown in Table 1. Individual stock solutions of each analyte (at 1 mg mL\textsuperscript{-1}) and a mixture of them were prepared in methanol. Working standard solutions were made by appropriate dilution in methanol and then stored in amber glass vials at −20 °C.

All organic solvents were HPLC or LC/MS grade and all other chemicals were analytical reagent grade. Ultrapure water was produced using a Milli-Q Gradient purification system from Merck Millipore (Bedford, MA, USA). Formic acid (98–100%), hydrochloric acid (37%), sodium hydroxide and anhydrous sodium carbonate were purchased from Merck (Darmstadt, Germany).

**Derivatization procedure**

Under optimized conditions, bisphenols were derivatized using PS chloride as follows: a standard solution of bisphenols was placed in a 1.8 mL glass vial, evaporated to dryness under a nitrogen flow and reconstituted in 200 μL of sodium carbonate buffer (50 mmol L\textsuperscript{-1}, pH 9.8). Then, 200 μL of 1 mg mL\textsuperscript{-1} PS chloride in acetonitrile were added and the vial was cap-sealed. After vortex-shaking for 10 s, the reaction mixture was placed in a dry block heater at 70 °C for 15 min. The reaction was stopped by cooling down on ice and 100 μL formic acid (1 mol L\textsuperscript{-1}) were added. The reaction mixture was passed through a 0.20 μm regenerated cellulose syringe filter and analyzed by LC/MS.

**LC/MS analysis**

Analyses were carried out on a binary high-pressure gradient LC system (Shimadzu Benelux, ’s-Hertogenbosch, The Netherlands) consisting of two LC-20AD pumps with a microvolume mixer and a DGU-20A degasser, an autosampler SIL-30AC and column oven CTO-30A. Chromatographic separation was performed on a reversed-phase Kinetex C18 column (100 × 2.1 mm, 2.6 μm; Phenomenex, Utrecht, The Netherlands), maintained at 50 °C. For the analysis of the derivatized bisphenols, mobile phases A and B were 0.1% formic acid in water (v/v) and 0.1% formic acid in methanol (v/v), respectively. The following linear gradient was used: 0 min, 40% B; 8.0 min, 88% B; 8.01 min, 40% B and 12.0 min, 40% B. For the underderivatized bisphenols, water and methanol were used as mobile phases A and B, respectively. The gradient used was as follows: 0 min, 10% B; 1.0 min, 40% B; 10 min, 100% B; 10.01 min, 10% B and 13.0 min 10% B. In both cases, the flow rate was set to 300 μL min\textsuperscript{-1} and the injection volume was 2 μL.

The LC system was coupled to an Orbitrap Elite hybrid ion trap-orbitrap mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) equipped with a heated electrospray ionization source (HESI-II). The ion transfer tube and the HESI vaporizer temperatures were both set at 300 °C. Nitrogen (boil-off) was employed as sheath gas, auxiliary gas and sweep gas at relative flow rates of 30, 15 and 1 arbitrary units, respectively. The HESI source was operated in positive mode for the derivatized bisphenols at a spray voltage of 3.5 kV and in negative ionization mode for the underderivatized bisphenols at 3.0 kV. The mass spectrometer performed a full FTMS scan event (m/z 100–1000, 15,000 FWHM resolution at m/z 400) and a subsequent data-dependent MS/MS event acquired at 30,000 FWHM.
resolution (at m/z 400). Precursor ions were fragmented by higher-energy collisional dissociation (HCD) with a normalized collision energy of 50% and an activation time of 100 ms. The precursor ion isolation width was 2 m/z units and dynamic exclusion was set to 2 with an exclusion time of 9 s. Quantitative experiments were performed in full scan mode (m/z 100–1000) under low-resolution conditions using the linear ion trap of the instrument. Data acquisition was controlled by Xcalibur 2.2 software (Thermo Fisher Scientific). External calibration for achieving high mass accuracy was carried out the day before the analysis according to the manufacturer’s guidelines. To prevent salts from entering the ion source, the LC eluate was diverted to waste during the first 1.5 min of the chromatographic run.

Statistical analysis
The construction and subsequent analysis of the response surface model, as well as all other statistical calculations, were carried out using the software package Statgraphics Centurion XV (Statpoint Technologies, Herndon, VA, USA).

RESULTS AND DISCUSSION

Preliminary experiments
The first experiments were conducted to evaluate if the previously reported conditions for the derivatization of BPA with PS chloride might be also suitable for the derivatization of its main analogues. The original procedure described by Xu[24] was slightly modified in order to increase its compatibility with both reversed-phase chromatography and the mass spectrometer. For instance, PS chloride solution was prepared in acetonitrile instead of acetone, and the buffer concentration was reduced from 100 mmol L\(^{-1}\) to 50 mmol L\(^{-1}\). Individual solutions of each bisphenol (500 ng mL\(^{-1}\) in methanol) were evaporated to dryness under a nitrogen flow and 200 μL of sodium carbonate buffer (50 mmol L\(^{-1}\); pH 10) were added, followed by 200 μL of PS chloride (1 mg mL\(^{-1}\) in acetonitrile). The mixtures were allowed to react at 60 °C for 5 min, cooled on ice and analyzed by LC/MS.

Under these conditions, the chromatograms showed the presence of major peaks corresponding to di-derivatized forms of bisphenols. In all cases, the full MS spectra displayed as base peaks the protonated molecules [M+H]\(^{+}\), which were in agreement with the exact m/z values within 0.5 ppm of mass measurement error (Table 2). One potential problem when derivatizing compounds with two derivatizable groups is that the reaction might lead to mixtures of mono- and di-substituted derivatives. For instance, derivatization of BPA with dansyl chloride has been reported to yield an important amount of mono-dansylated BPA when the derivatization reaction is carried out under non optimized conditions.[22] After derivatization with PS chloride, we examined the chromatograms for the mono-derivatized bisphenols (Supplementary Table SI, Supporting Information), which were also present, but to a much lower extent than the di-derivatized forms (data not shown). Although derivatization of phenols with both dansyl chloride and PS chloride is based on a similar nucleophile substitution reaction, the smaller size of the PS group may positively affect the derivatization yield. Thus, the lower steric hindrance of the mono-PS derivative would increase the derivatization rate of the second hydroxyl moiety of bisphenols.

Fragmentation of pyridine-3-sulfonyl derivatives
Derivatization reagents used for LC/ESI-MS should enhance the ESI response by attaching a readily ionizable group to the original molecule.[17,18] Nevertheless, most of them provide only reagent-specific ions upon MS/MS fragmentation of the corresponding [M+H]\(^{+}\) ions, which usually results in numerous co-eluting interferences when analyzing complex samples.[28]

Thus, the MS/MS fragmentation patterns of the [M+H]\(^{+}\) ions of the di-substituted PS derivatives were studied in order to evaluate the analyte-specificity of the obtained product ions. Taking advantage of the high-resolution/accurate-mass measurements of the Orbitrap, the main product ions could be tentatively identified with mass measurement errors below 2 ppm (Table 2). MS/MS experiments were carried out by HCD. During HCD fragmentation, the precursor ions are fragmented in a collision cell rather than an ion trap and then transferred back through the C-trap into the Orbitrap for analysis at high resolution. Thus, HCD fragmentation patterns have been found to be similar to those obtained in triple quadrupole instruments.[29] This would allow the information gathered to be transferred and applied to quantitative analysis by MRM.

The product ion spectrum of protonated BPA-diPS showed a base peak at m/z 354.0792, which was assigned to the product ion resulting from the loss of one methyl group and one PS moiety (Fig. 1(a)). Two other abundant ions were obtained at m/z 290.1175 and 212.0833. The former was proposed as the product ion produced by the loss of SO\_(2\) (63.9619 u) from the ion at m/z 354.0792 via rearrangement of the sulfonyl group (Scheme 1(a)). Elimination of SO\_(2\) via rearrangement has been previously observed in the product ion spectra of PS derivatives of estrogens,[27] dansyl derivatives of some phenolic compounds,[30] and some aromatic sulfonamides.[31,32]

The ion at m/z 212.0833 was assigned to a radical cation derived from the ion at m/z 354.0792 via homolytic cleavage of the S–O bond between PS and the BPA ring (Scheme 1(b)). The calculation of the ring and double bond equivalents (RDB) for this product ion showed an integer value (Table 2), which is in agreement with it being an odd-electron cation. The formation of this radical product ion is presumably attributed to its stabilization through conjugation by the adjacent aromatic ring. Odd-electron dissociations, which involve homolytic bond cleavages to yield radical product ions, are commonly observed in electron ionization (EI)-MS, but they are less common in collision-induced dissociation under ESI and atmospheric pressure chemical ionization (APCI) conditions.[33,34] Several exceptions to the ‘even-electron rule’ have, however, been reported to involve radical eliminations leading to odd-electron product ions of high stability.[35,36] Other less abundant product ions were obtained after the initial cleavage of the C–C bond between the methylene group and one of the aromatic rings. Among them, the product ion at m/z 134.0726 was also identified...
as a radical cation. These results are consistent with the findings obtained by low-resolution mass spectrometry after collision-induced dissociation (CID) fragmentation of the [M + H]^+ ion of di-derivatized BPA.[24,26]

Most of the studied bisphenols followed a fragmentation pathway similar to that of BPA (Figs. 1(a)–1(c) and Supplementary Fig. S1, Supporting Information), usually initiated by loss of one substituent at the methylene position.

### Table 2. Retention times (t<sub>R</sub>), accurate and exact m/z, proposed formulae, relative mass measurement errors (Δm) and ring and double-bond equivalents (RDB) of the di-derivatized bisphenols and their product ions

| Compound | t<sub>R</sub> (min) | Accurate m/z | Formula | Exact m/z | Δm (ppm) | Product ions (m/z) | Formula | Δm (ppm) | RDB |
|----------|---------------------|--------------|---------|-----------|-----------|---------------------|---------|-----------|------|
| BPS-diPS | 3.91                | 533.0141     | [C<sub>22</sub>H<sub>17</sub>N<sub>2</sub>O<sub>8</sub>S<sub>3</sub>]<sup>+</sup> | 533.0142 | -0.188    | [C<sub>17</sub>H<sub>12</sub>O<sub>2</sub>N]<sup>+</sup> | -0.89   | 12        |
|          |                     |              |         |           |           | [C<sub>11</sub>H<sub>9</sub>O<sub>3</sub>N]<sup>+</sup> | 1.17    | 8.5       |
|          |                     |              |         |           |           | [C<sub>12</sub>H<sub>5</sub>O<sub>3</sub>NS]<sup>+</sup> | 1.16    | 8.5       |
| BPF-diPS | 5.08                | 483.0680     | [C<sub>23</sub>H<sub>19</sub>N<sub>2</sub>O<sub>6</sub>S<sub>2</sub>]<sup>+</sup> | 483.0679 | 0.207     | [C<sub>18</sub>H<sub>13</sub>O<sub>2</sub>N]<sup>+</sup> | -0.90   | 12        |
|          |                     |              |         |           |           | [C<sub>13</sub>H<sub>10</sub>O<sub>2</sub>N]<sup>+</sup> | 0.19    | 12.5      |
| BPE-diPS | 5.5                 | 497.0835     | [C<sub>24</sub>H<sub>21</sub>N<sub>2</sub>O<sub>6</sub>S<sub>2</sub>]<sup>+</sup> | 497.0836 | -0.201    | [C<sub>18</sub>H<sub>14</sub>O<sub>2</sub>N]<sup>+</sup> | -0.12   | 12        |
| BPA-diPS | 5.93                | 511.0991     | [C<sub>25</sub>H<sub>23</sub>N<sub>2</sub>O<sub>6</sub>S<sub>2</sub>]<sup>+</sup> | 511.0992 | -0.196    | [C<sub>19</sub>H<sub>16</sub>O<sub>2</sub>N]<sup>+</sup> | -0.64   | 12.5      |
| BPB-diPS | 6.38                | 525.1148     | [C<sub>26</sub>H<sub>25</sub>N<sub>2</sub>O<sub>6</sub>S<sub>2</sub>]<sup>+</sup> | 525.1149 | -0.190    | [C<sub>20</sub>H<sub>18</sub>O<sub>2</sub>N]<sup>+</sup> | 0.09    | 8.5       |
| BPAF-diPS | 6.62               | 619.0426     | [C<sub>27</sub>H<sub>27</sub>F<sub>6</sub>N<sub>2</sub>O<sub>6</sub>S<sub>2</sub>]<sup>+</sup> | 619.0427 | -0.162    | [C<sub>21</sub>H<sub>20</sub>O<sub>2</sub>N]<sup>+</sup> | 0.00    | 8.5       |
| BPAP-diPS | 6.9                | 573.1147     | [C<sub>30</sub>H<sub>25</sub>N<sub>2</sub>O<sub>6</sub>S<sub>2</sub>]<sup>+</sup> | 573.1149 | -0.349    | [C<sub>23</sub>H<sub>18</sub>O<sub>2</sub>N]<sup>+</sup> | 0.18    | 16.5      |
| BPZ-diPS | 6.94                | 551.1307     | [C<sub>28</sub>H<sub>27</sub>N<sub>2</sub>O<sub>6</sub>S<sub>2</sub>]<sup>+</sup> | 551.1305 | 0.363     | [C<sub>22</sub>H<sub>20</sub>O<sub>2</sub>N]<sup>+</sup> | -0.21   | 13.5      |
| BPP-diPS | 8.04                | 629.1778     | [C<sub>34</sub>H<sub>33</sub>N<sub>2</sub>O<sub>6</sub>S<sub>2</sub>]<sup>+</sup> | 629.1775 | 0.477     | [C<sub>27</sub>H<sub>22</sub>O<sub>2</sub>N]<sup>+</sup> | -0.18   | 16.5      |

Δm = mass measurement error; RDB = ring and double bond equivalents

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probably driven by the heterolytic S–O bond cleavage between one PS moiety and the corresponding phenol. An alternative common fragmentation route started with the cleavage of the C–C bond between the methylene carbon and one of the rings of the bisphenol backbone. For BPS, BPF, and BPZ, the initial fragmentation pathway was slightly different (Figs. 2(a)–2(c)). The MS/MS spectrum of BPS-diPS showed a base peak at \( m/z \) 327.0557, which was assigned to a radical cation formed from the \([\text{M+H}]^+\) ion by homolytic cleavage of the S–O bond between one PS moiety and the phenolic ring. Subsequent fragmentations of this ion yielded even-electron product ions at \( m/z \) 218.0273 and 170.0602. The former might be obtained via a sulfone-sulfinate rearrangement followed by the cleavage of the resulting S–O bond, as previously reported for some diaryl sulfones under EI conditions.\(^{[37]}\) The ion at \( m/z \) 170.0602 would be formed via heterolytic cleavage of the C–S bond. For BPF-diPS and BPZ-diPS, major product ions were obtained by the loss of the two PS moieties from the \([\text{M+H}]^+\) ions, yielding product ions at \( m/z \) 199.0754 and 267.1382, respectively.

**Figure 1.** High-resolution ESI-MS/MS spectra obtained by higher-energy collisional dissociation with a normalized collision energy of 50% and proposed fragmentation pathways for the \([\text{M+H}]^+\) ions of the PS di-derivatized forms of BPA (a), BPE (b), and BPB (c).
Scheme 1. Proposed mechanisms for (a) the elimination of SO₂ and (b) the formation of the odd-electron ions from the PS derivatives of bisphenols in ESI-MS/MS.

Figure 2. High-resolution ESI-MS/MS spectra obtained by higher-energy collisional dissociation with a normalized collision energy of 50% and proposed fragmentation pathways for the [M+H]+ ions of the PS di-derivatized forms of BPS (a), BPF (b), and BPZ (c).
Secondary fragmentations involving the loss of SO₂ via the proposed rearrangement of the sulfonyle group were observed for all the derivatized bisphenols. In addition, all the compounds showed at least one odd-electron product ion (Table 2), suggesting that the loss of the PS moiety by the proposed homolytic cleavage might be a usual process for those cases where the resulting product ions are resonance-stabilized.

As shown, fragmentation of the protonated PS derivatives of bisphenols yields highly informative MS/MS spectra presenting abundant analyte-specific product ions. The information obtained would permit a more rational selection of specific product ions for MRM acquisition in quantitative analysis of these compounds, minimizing potential interferences in complex matrices. For instance, derivatization of BPA with dansyl chloride leads to a derivative whose [M + H]⁺ ion yields product ions at m/z 171 and 156, derived from the protonated dimethylaminonaphthyl moiety of the reagent [21,22,38] and therefore common to most of compounds derivatized with the same reagent.

**Optimization of derivatization conditions: response surface design**

Once the identity and suitability of the bisphenol derivatives had been confirmed, a response surface experimental design was applied to determine the optimal values for the main variables potentially affecting the derivatization yield. This approach allows predictive models to be obtained that accurately describe changes in the responses within the experimental region. A Box–Behnken experimental design (BBD) with three center points was selected, which allowed the effects of three factors to be evaluated, at three levels each, involving 15 experiments and allowing 5 degrees of freedom to estimate the experimental error. All experiments were performed in randomized order to minimize the effects of uncontrolled variables that might introduce bias on the outcome.

Sulfonyl chlorides are known to undergo a nucleophilic substitution reaction with phenols, where the electron-withdrawing chlorine induces an electron-deficient center at the tetra-coordinated sulfur atom holding the halogen atom, thereby facilitating the nucleophilic attack at the sulfur atom. This mechanism can be promoted under basic conditions via deprotonation of the phenol, increasing its nucleophilicity. On the other hand, a too high pH value might lead to undesirable hydrolysis of the resulting sulfonic esters. Considering the pKa values of the studied bisphenols (7.42–10.45, Table 1), it was decided to evaluate the effect of the buffer pH in the range of 9.80 to 11.20. Temperature and reaction time are also important parameters in this kind of reaction, and therefore they were included in the response surface model. The considered factors as well as their corresponding levels and identification codes are given in Supplementary Table S2 (Supporting Information). The obtained responses (relative peak areas of the derivatized bisphenols) were fitted to a polynomial quadratic model and then analyzed by analysis of variance (ANOVA). Table 3 summarizes the numerical values of the standardized main effects, as well as their interactions and quadratic terms. These values were obtained by dividing the estimated effect of each factor or interaction by its standard error. The absolute value of each effect is proportional to the influence of the associated factor on the derivatization yield. A positive sign indicates an improvement in reaction yield when the factor varies, within the domain of the experimental design, from the low to the high level, and a negative one indicates the opposite trend. The fitted quadratic models were assessed with the adjusted coefficient of determination (R²), which presented values between 0.7579 and 0.9414, revealing that the experimental data were in good agreement with the values predicted by the constructed models.

As shown in Table 3, the temperature was the most important factor presenting statistical significance (95% confidence level) for nine of the ten bisphenols, with the highest responses corresponding to 70 °C, as deduced from the positive sign of its standardized effect. The buffer pH data suggested two different trends. For the most acidic bisphenols, i.e. BPS and BPAF, the pH of the buffer had a significant negative impact on the derivatization yield, whereas for the other compounds this factor was not significant. This result might be attributed to a lower hydrolytic stability of these sulfonate esters at higher pH values. Thus, during alkaline hydrolysis the leaving group is normally related to the pKa of the anion – the conjugate acid, whereas for the other compounds this factor was not significant. This result might be attributed to a lower hydrolytic stability of these sulfonate esters at higher pH values. Thus, during alkaline hydrolysis the leaving group must be able to stabilize the resulting negative charge. This is normally related to the pKa of the anion’s conjugate acid, with lower pKa values being associated with better leaving

| Compound | A:pH | B:Temp | C:Time | AB | AC | BC | AA | BB | CC | R² |
|----------|------|--------|--------|----|----|----|----|----|----|----|
| BPS      | −3.4*| 3.7*   | 0.3    | −0.7| −1.1| 0.7 | 3.4*| 0.9 | 1.1 | 0.8895 |
| BPF      | 1.4  | 5.5*   | −0.2   | 0.5 | −0.4| −0.8| 0.0 | −0.6| −1.0| 0.8744 |
| BPE      | −0.6 | 8.0*   | −0.5   | 2.0 | −0.8| −0.3| 1.6 | −2.5| −1.4| 0.9414 |
| BPA      | −0.3 | 4.9*   | −0.9   | 1.0 | −0.5| 0.5 | 0.8 | −1.2| −0.6| 0.8516 |
| BPB      | 0.6  | 3.5*   | −1.2   | 1.0 | −1.9| −0.9| 0.1 | −1.3| 0.1 | 0.8108 |
| BPAF     | −5.1*| 2.5    | −2.5   | 1.2 | 0.6 | 0.6 | 3.0*| 0.1 | 1.0 | 0.9090 |
| BPAP     | −1.3 | 2.8*   | −1.7   | 1.1 | 0.3 | 0.3 | 0.9 | −0.8| 0.1 | 0.7579 |
| BPZ      | 0.0  | 5.3*   | −2.3   | 2.0 | 0.4 | −0.5| −0.6| −1.9| −0.3| 0.8920 |
| BPP      | 1.6  | 4.5*   | −1.9   | 1.4 | −0.6| −0.8| 0.6 | −2.7*| −0.2| 0.8825 |

*Statistically significant (p < 0.05)
group ability. Quadratic terms associated with the pH (AA) also presented statistically significant effects for these compounds, suggesting a non-linear variation in the efficiency of derivatization within the domain of the design. Neither the derivatization time nor the two-factor interactions had any statistically significant effect on the reaction yield. Three-dimensional response surface plots showing the effect of these factors on the derivatization for several representative bisphenols are depicted in Fig. 3.

In order to establish the optimum conditions for the derivatization of the whole set of bisphenols, a desirability function was employed. This approach transforms each response into a scale-free variable known as desirability (di). The individual desirabilities predicted by the Box–Behnken design are then combined using a weighted geometric mean to obtain the overall desirability (D). Thus, a multi-response optimization is converted into single-response optimization. The scale of the individual desirability functions ranges from di = 0, for a completely undesired response, to di = 1 for a fully desired response. A higher weight was assigned to BPA and those bisphenols with higher reported occurrence, namely BPF, BPS and BPAF. The overall desirability function produced a maximum value (D = 0.79) at pH 9.8, 70 °C and 15 min. These conditions were selected as the final conditions for the derivatization of bisphenols with PS chloride. Xu[24] reported as optimal conditions for the derivatization of BPA pH 10, 60 °C and 5 min reaction time, suggesting the partial hydrolysis of the di-derivatized form at longer times to yield the mono-derivatized BPA. Nevertheless, our results showed that even at 70 °C only traces of mono-derivatized bisphenols could be detected in the chromatograms, indicating that the derivatization reaction with PS chloride was almost quantitative under the proposed optimized conditions. However, in order to avoid potential hydrolysis of the derivatized bisphenols, 100 μL of formic acid (1 mol L−1) were added to the reaction solution at the end of the reaction time, which lowered the pH of the solution to around pH 3.

Stability of pyridine-3-sulfonyl derivatives

Information on the stability of the derivatives is crucial for the design of analysis sequences and for deciding on the shelf life of calibration solutions. The stability of the PS derivatives was evaluated by testing the influence of freeze (−20 °C) and thaw (22 ± 2 °C) cycles, short-term exposure to room temperature and long-term exposure to −20 °C. For the freeze/thaw cycle stability test, a 10 ng mL−1 mix of bisphenols was derivatized as previously described and three aliquots were stored at −20 °C for 24 h and then thawed at room temperature. When completely thawed, the aliquots were refrozen for 12 h under the same conditions. The freeze/thaw cycle was repeated five times. The short-term room temperature stability was assessed by analyzing three aliquots of the derivatized mix, which were kept on the bench for 16 h at room temperature (22 ± 2 °C). This period corresponds to the maximum time that the samples stay in the autosampler during a sequence. The long-term stability at −20 °C was evaluated by analyzing three aliquots of the PS derivatives mix, which were kept at −20 °C over 7 days.

Upon completion of the stability tests, all the solutions were analyzed and the obtained peak areas were compared with the area obtained for a freshly derivatized solution.

Figure 3. Response surface plots showing the influence of temperature and pH on the derivatization yield of several representative bisphenols.
The stability of bisphenol derivatives was demonstrated by Student’s t-test, showing in all cases p-values above 0.05 (Table 4).

**Signal improvement**

To evaluate the effect of derivatization on the signal abundance, a solution of bisphenols (10 ng mL\(^{-1}\)) was analyzed before and after derivatization with PS chloride. For the underivatized compounds, the analysis was carried out in negative ESI mode using water and methanol as mobile phases as suggested in the literature.\[^{11,13,14,39}\] A comparison of the signal-to-noise (S/N) ratios obtained for all the studied compounds is shown in Fig. 4. The corresponding extracted ion chromatograms for the derivatized and underivatized bisphenols can be seen in Supplementary Fig. S2 (Supporting Information). In order to obtain a more realistic estimation of S/N ratio, these experiments were performed under low-resolution conditions using the linear ion trap of the MS instrument. The results showed an increase in the S/N ratio for all the derivatized bisphenols except for BPS and BPAF. The highest improvement was obtained for BPA with around 50-fold higher S/N ratio than for the underivatized form, whereas BPP showed an 8-fold higher S/N ratio after derivatization. These results are consistent with those obtained by Patterson et al.,\[^{25}\] who reported a 20-fold increase in sensitivity after derivatization with PS chloride for the analysis of BPA in rhesus monkey serum samples by MRM. On the contrary, for BPS and BPAF, the S/N ratio was decreased by nearly one-half compared with their underivatized forms. This different behavior may be, in part, explained by the higher acidity of these compounds and the higher charge delocalization of the corresponding anions, which would lead to higher ionization efficiency under negative ESI than for the other bisphenols. However, this relatively small decrease in sensitivity is compensated for by the great improvement observed for the other bisphenols after derivatization. The obtained results are in good agreement with the general understanding of the ESI process.\[^{40}\] Thus, the introduction of a PS functional group

**Table 4. Stability tests of the derivatized bisphenols under different conditions**

| Compound | Freeze/thaw cycles | Long-term freeze | Short-term room temperature |
|----------|--------------------|------------------|-----------------------------|
| BPS      | RSD (%) 2.3        | 3.1              | 2.9                         |
| p-value  | 0.058              | 0.098            | 0.230                       |
| BPF      | RSD (%) 1.6        | 1.8              | 3.8                         |
| p-value  | 0.438              | 0.154            | 0.061                       |
| BPE      | RSD (%) 6.1        | 1.8              | 2.4                         |
| p-value  | 0.343              | 0.127            | 0.056                       |
| BPA      | RSD (%) 1.3        | 2.0              | 2.2                         |
| p-value  | 0.445              | 0.062            | 0.052                       |
| BPB      | RSD (%) 2.6        | 2.5              | 2.1                         |
| p-value  | 0.115              | 0.108            | 0.056                       |
| BPAF     | RSD (%) 3.7        | 3.2              | 1.7                         |
| p-value  | 0.190              | 0.065            | 0.174                       |
| BPAP     | RSD (%) 1.2        | 1.5              | 4.6                         |
| p-value  | 0.135              | 0.056            | 0.180                       |
| BPZ      | RSD (%) 2.3        | 2.5              | 1.8                         |
| p-value  | 0.076              | 0.083            | 0.164                       |
| BPP      | RSD (%) 1.6        | 7.2              | 4.1                         |
| p-value  | 0.252              | 0.081            | 0.072                       |

**Figure 4.** Comparison of signal-to-noise ratios for a mix of bisphenols (10 ng mL\(^{-1}\)) without derivatization (ESI–) and after derivatization with PS chloride (ESI+).
Figure 5. Overlapped extracted ion chromatograms of the PS di-derivatized bisphenols (10 ng mL\(^{-1}\)) in ESI+. BPS, \(m/z\) 533.01; BPF, \(m/z\) 483.07; BPE, \(m/z\) 497.08; BPA, \(m/z\) 511.10; BPB, \(m/z\) 525.11; BPFA, \(m/z\) 619.04; BPAP, \(m/z\) 573.11; BPZ, \(m/z\) 551.13; BPP, \(m/z\) 629.18.

bearing a basic nitrogen shifted the predicted pKa of the molecule to around 2.5 (pKa of the conjugate acid form of the basic amine), which enhanced the ionization efficiency of the bisphenols derivatives under acidic conditions. Figure 5 shows the overlapped extracted ion chromatograms obtained for the studied bisphenols under optimized conditions.

CONCLUSIONS

Derivatization of BPA and its structural analogues with pyridine-3-sulfonyl chloride is proposed as a specific, sensitive, high-throughput approach for their analysis by LC/ESI-MS. To the best of our knowledge this derivatization reagent has been applied for the first time in the present work to bisphenols other than BPA. A response surface experimental design identified the derivatization temperature and the buffer pH as the most important factors affecting the derivatization yield of bisphenols. Optimized derivatization conditions allowed di-substituted derivatives to be obtained quantitatively for all the bisphenols. The introduction of readily ionizable moieties in their structures led to most of the derivatized bisphenols showing significant improvements in their signal-to-noise ratios compared with the underivatized forms. Their fragmentation pathways were studied by high-resolution/accurate-mass Orbitrap mass spectrometry, showing abundant analyte-specific product ions suitable for use in quantitative analysis by MRM. The stability of the bisphenol derivatives was demonstrated through several freeze/thaw cycles, short-term room temperature and long-term cold storage.

The proposed derivatization procedure provides a convenient way to improve the detection capabilities of bisphenols by LC/ESI-MS in applications requiring both high sensitivity and selectivity. For the future, it is planned to integrate the presented derivatization procedure into analytical methods for the determination of bisphenols in a variety of complex food matrices and to demonstrate its applicability to routine analysis by LC/MS/MS.

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