

# *Mass Spectra of Sulfonephthalein pH Indicator Dyes and Their Impurities*

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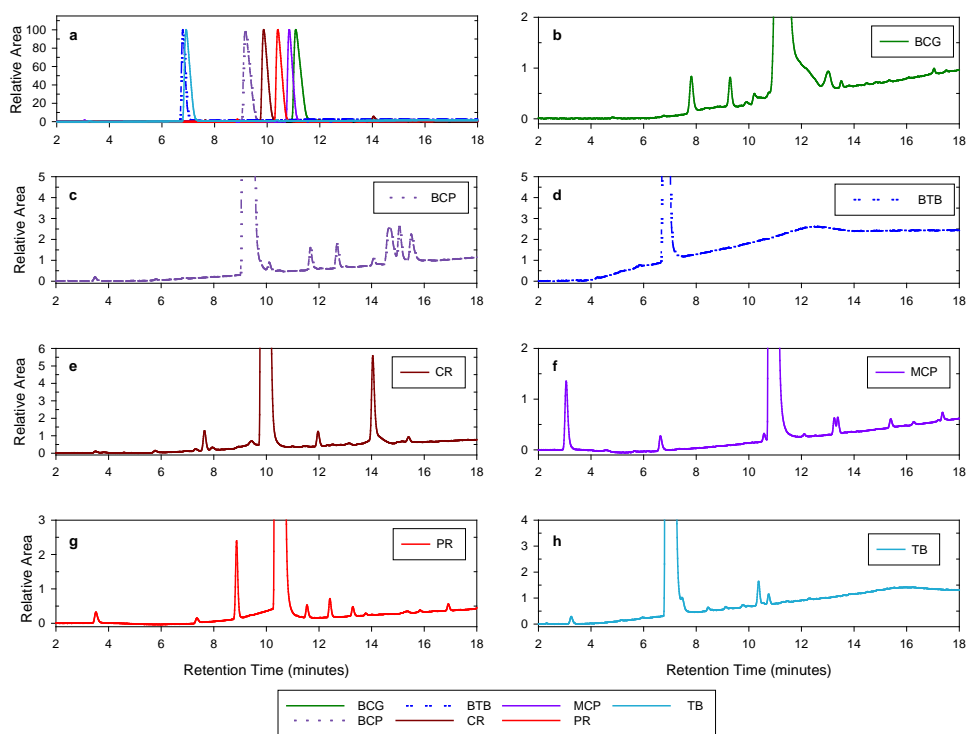
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## 1. Summary

Sulfonephthalein pH indicator dyes have historically been noted for impurities originating from the synthesis process, which lead to batch to batch differences in pH measurements [1]. Uncertainties up to 0.1 pH units have been attributed to impurities in these reagents [2]. In an effort to understand the extent of impurities in these dyes and the potential impact on the expanded uncertainty of spectrophotometric pH measurements, we have examined seven commercially available sulfonephthalein pH indicators using liquid chromatography-mass spectrometry (LC-MS): bromocresol green (BCG), bromocresol purple (BCP), bromothymol blue (BTB), cresol red (CR), meta-cresol purple (MCP), phenol red (PR), and thymol blue (TB). Peaks eluting from the LC (Fig. 1) were subjected to fragmentation by mass spectrometry. The resulting dataset will aid in efforts to improve the synthesis or purification of these dyes [3].

## 2. Data Specifications

<b>NIST Operating Unit(s)</b>	Material Measurement Laboratory; Chemical Sciences Division; Inorganic Measurement Science and Organic Chemical Measurement Science Groups
<b>Format</b>	Proprietary Thermo Scientific MS datafiles (*.raw) have been converted into ANDI-MS (ASTM E1947) NetCDF format (*.cdf)
<b>Instrument</b>	Thermo Scientific Q-Exactive Quadrupole-Orbitrap Mass Spectrometer
<b>Spatial or Temporal Elements</b>	Data collection date 2015-11-25
<b>Data Dictionary</b>	<a href="http://www.unidata.ucar.edu/software/netcdf/">http://www.unidata.ucar.edu/software/netcdf/</a> <a href="http://www.astm.org/Standards/E1947.htm">http://www.astm.org/Standards/E1947.htm</a>
<b>Accessibility</b>	All datasets submitted to <i>Journal of Research of NIST</i> are publicly available.
<b>License</b>	<a href="https://www.nist.gov/director/licensing">https://www.nist.gov/director/licensing</a>
<b>Hash Value</b>	See <a href="https://doi.org/10.18434/M3JP40">https://doi.org/10.18434/M3JP40</a>



**Fig. 1.** Liquid chromatograms of sulfonephthalein dyes. Figure 1a shows an overlay with all the chromatograms taken. Figures 1b-h show impurities in each dye at the visible wavelengths used to determine pH. Each impurity peak underwent sequential fragmentation.

### 3. Methods

Each sample was prepared by dissolving 25 mg in deionized water (resistivity of  $18.2 \text{ M}\Omega \cdot \text{cm}$  at  $25^\circ\text{C}$ ) filtered through  $0.22 \mu\text{m}$  to yield a solution near  $10 \text{ mmol/L}$ . Samples were filtered with  $0.45 \mu\text{m}$  nylon filters and were then run in triplicate on an UltiMate 3000 Liquid Chromatograph with a Q-Exactive Quadrupole-Orbitrap Mass Spectrometer. A linear gradient program was used which changed the composition of solvent from 5 % volume fraction methanol: 95 % volume fraction 5 mmol/L formate/formic acid buffer (adjusted to pH 3.5) to 95 % volume fraction methanol: 5 % volume fraction 5 mmol/L formate/formic acid buffer (adjusted to pH 3.5) over a 40 min period at a constant flow rate of  $0.3 \text{ mL/min}$ . The following chromatographic conditions were used:

Column: MAC-MOD Analytical, Halo C18 column,  $2.1 \text{ mm ID} \times 100 \text{ mm length}$   
 Ionization: Electrospray Ionization (positive and negative mode alternating)  
 Injection volume:  $10 \mu\text{L}$   
 Spray Voltage (V):  $3000(+)/2500(-)$   
 Capillary Temperature:  $350^\circ\text{C}$   
 Sheath Gas (relative flow rate): 35  
 Aux Gas (relative flow rate): 10  
 Probe Heater Temperature:  $300^\circ\text{C}$   
 Scan: MS1, data-dependent-MS2 (TopN)  
 Resolution: 70 000 (measured at  $m/z$  200 by FWHM)  
 Scan Range:  $m/z$  80 to  $1000^1$

<sup>1</sup> Certain commercial equipment, instruments, or materials are identified in this poster to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

#### 4. Impact

These findings will be helpful in ongoing investigations examining the purity of these dyes and their application to ocean and fresh water pH studies.

#### 5. References

- [1] Yao W, Liu X, Byrne RH (2007) Impurities in indicators used for spectrophotometric seawater pH measurements: Assessment and remedies. *Marine Chemistry* 107:167-172. <https://doi.org/10.1016/j.marchem.2007.06.012>
- [2] Liu X, Patsavas MC, Byrne RH (2011) Purification and characterization of meta-cresol purple for spectrophotometric seawater pH measurements. *Environ. Sci. Technol* 45:4862-4868. <https://doi.org/10.1021/es200665d>
- [3] Tillu VA, Dumbre DK, Borate HB, Wakharkar RD, and Choudhary VR (2012) Solvent-free one-pot synthesis of sulfonephthaleins from saccharin and phenols. *Synthetic Communications* 1101-1107. <https://doi.org/10.1080/00397911.2010.535946>

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