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Letter to the Editor

Critique of the article "Preparation and application of 2,4,6-tribromo-[$^{13}C_6$]-anisole for the quantitative determination of 2,4,6-tribromoanisole in wine" by Giannikopoulos and Whitfield

Sir

I refer to a recent publication (*Food Chemistry, 113* (2009) 307–312) in your Journal by Giannikopoulos and Whitfield in which they describe an analytical method for the analysis of the known taint 2,4,6-tribromoanisole (TBA) in wine. Their method uses solid phase microextraction to analyse for this compound by gas chromatography-mass selective detection and 2,4,6-tribromo-1-methoxy-[$^{13}C_6$]benzene as an internal standard to measure the amount of TBA in their spiked red and white wines.

I was curious as to why these authors had presented the results of their experiments in a format that I had not previously encountered in a peer-reviewed analytical journal. Rather than reporting the calculated amounts of TBA in their samples, Giannikopoulos and Whitfield reported the relative detector responses that were obtained from the analysis of their seven white wine and eight red wine samples, both of which were in the range of 2–250 ng L^{-1} and the two series otherwise appeared identical expect that the white wine samples did not include an 8 ng L^{-1} sample. Furthermore, the data, which were used to calculate relative standard deviations, i.e. variation of the method at the various concentrations, were summarized in the article as the mean and standard deviation of the "best two results obtained" from "experiments [that] were conducted in triplicate". Consequently, the calculated relative standard deviations that are reported in the article *must be interpreted with caution because the actual standard deviations and, therefore the imprecision of the method at the various concentrations, will, arguably, be higher than those reported* by the authors.

The rejection of experimental data for the purpose of statistical analysis can only be justified when an appropriate and acceptable test demonstrates the values are indeed deviant or when a systematic error has been identified in the questionable data. The so-called Dixon *Q* test (Rorabacher, 1991) is frequently used for this purpose. In the case where a data set has three values, i.e. three degrees of freedom, then the critical value *Q* at the 95% confidence interval for a two tail test is 0.970 (Rorabacher, 1991). If the calculated *Q* value for a particular data point exceeds the critical value, then it can be considered to be an outlier and justifiably excluded, *but not eliminated from the data set*, from any data analysis. Consequently, for a *Q* value of 0.97 the rejection of data can be justified if one of the data points (x_1) tends towards zero and the ratio of the middle value (x_2) to the third value (x_3) (when the data are arranged such that $x_1 < x_2 < x_3$) is greater than 0.97 or the difference between the two retained data points tends towards zero. Alternatively, a *Q* value of greater than 0.97 can also be achieved when the third data point (x_3) is much larger than the two retained data points. In the absence of a systematic error, then it is reasonable to assume that the data points that were rejected by the authors within triplicate sets were not drastically different to those that were retained. In this situation the rejection of a data point can be justified when the difference between the two "best" values tends towards zero. An inspection of the data reported in the manuscript reveals that this is not the case for the majority of the best pair values and, therefore, the rejection of data in these cases cannot be justified on the basis of the Dixon test. The arbitrary rejection of data, if for the sole purpose of improving the quality of a reported parameter, is inappropriate, (Resnik, 2000) and in cases where the rejected data conceal or reduce the extent of problems with research design, instrumental error, possible biases, or methodology is even more problematic (Resnik, 1998).

A further analysis of the data reveals that the expected and calculated (which were not reported) amounts of TBA in white wine are in the range of 73–300% and in the range of 70–450% for red wines based on their reported calibration curves and the data shown in Tables 1–4. It would be well to note that the calibration curves that were generated by the authors were forced through the origin. Replotting the red wine data without forcing the curve through the origin shows that the *x*, y intercepts for the 352/344 ion pair data set are −3.6 ng L^{-1}, 0.16, respectively, and −3.0 ng L^{-1}, 0.13 for the 352/346 ion pair data set. Such *x*, *y* intercepts are typical of calibration curves that are obtained from the analysis of samples in which the analyte is a component of the matrix and the concentration is increased by addition of the analyte to the sample, i.e. the standard addition technique. It seems feasible then to suggest that the stock red wine sample was either contaminated with TBA or, more likely, that there was interference from a chromatographically unresolved, co-extracted compound or compounds. Performing the replotting exercise with the white wine data did not show the same trend in that the curves intersected the *x*, *y* axes before and below (respectively) the origin. However, when the data were replotted after excluding the first (2 ng L^{-1}) and last (250 ng L^{-1}) data points, the curves were consistent with those obtained from replotting the red wine data. It may be the case that the actual variation in these two data points is much higher than that reported in the article and so they skew the curve or the consistency of trend after rejecting these two points is fortuitous. The reason for the significant *y* intercept for the red wine calibration data is unclear because the authors did not provide selectivity data in the way of ion ratios and, more importantly, did not provide data from the analysis of the red wine before adding TBA to the samples. This is also the case for the white wine data.

Perhaps the most puzzling aspect of the article is the reported limit of detection of TBA in red wine. The authors claim the method is capable of detecting 2 ng L^{-1} based on a "minimum signal to noise response [sic] greater than three times the background noise". However, it is difficult to appreciate how this limit of detection can be achieved in red wines by the reported method because the detector appears to be unresponsive to TBA when its concentration is below 8 ng L^{-1}. *Indeed, the relative response decreases by*

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25% when the concentration of TBA in the red wine samples is increased from 2 to 4 ng L^{-1}. An inspection of the red wine data indicates that the detector begins to respond to the analyte in the expected manner when the concentration of TBA in red wine is between 8 and 16 ng L^{-1}.

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