

BRIEF REPORT

Screening check test to confirm the relative reactivity and applicability of 2,4-dinitrophenylhydrazine impregnated-filters for formaldehyde on other compounds

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Abstract

Objectives: A simple check test method was designed to confirm whether a 2,4-dinitrophenylhydrazine (DNPH) filter for formaldehyde can be used to measure other compounds.

Methods: Sample mixtures containing the same concentrations of formaldehyde, acetaldehyde, and acetone were spiked to the DNPH-filter, extracted, and then measured using high performance liquid chromatography with photodiode array detector (HPLC-PDA). The amounts of DNPH-derivatives versus the amounts of spiked samples were then plotted.

Results: When the amount of DNPH \ll the total amount of spiked samples, the amount of DNPH-derivatives was formaldehyde $>$ acetaldehyde \gg acetone. This order corresponded to the relative rate constants for the reaction. Therefore, this study confirmed that acetone was not collected at the formaldehyde sampling rate.

Conclusions: This check test easily measured the reaction rate order and can be used as a simple test to determine whether other samples can be measured by the analytical methods used for the specified sample.

KEYWORDS

aldehyde, DNPH, ketone, passive sampler, reactivity screening

1 | INTRODUCTION

Formaldehyde is carcinogenic to humans; therefore, its presence in workplace air needs to be monitored to determine if exposure controls are adequate.¹ To sample air within the workplace, 2,4-dinitrophenylhydrazine (DNPH)-impregnated silica gel (or filters) are generally employed as a collection and derivatization agent, for both the passive and active sampling of formaldehyde.²⁻⁴

2,4-dinitrophenylhydrazine has been also used for the sample analysis of other aldehyde and ketone because DNPH reacts not only with formaldehyde but also other aldehyde and ketones to produce hydrazone.⁵ The DNPH on the filter reacts very quickly with compounds in the air.

Formaldehyde is not the only hazardous compound that needs to be measured in workplaces. Generally, it is preferable if many hazardous target compounds can be measured by fewer analytical methods than the number

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of target compounds. That is, in the case of hazardous compounds in workplaces where formaldehyde needs to be monitored, it would be best if many compounds could be measured by the DNPH method. However, the reaction rates of hazardous compounds with DNPH are different, depending on the compounds.⁶ Furthermore, slight differences in the derivatization conditions can affect the reactivity and yield.⁷ Therefore, it would be desirable if the DNPH method for formaldehyde could also be used for the other compounds. It is therefore necessary to confirm whether these other compounds can be measured by the DNPH analytical method.

The reaction rate and relative reaction rate have both been used to evaluate the reactions of various compounds.^{8–14} These methods for reaction rates could be used to confirm whether other compounds are able to be measured by the analytical methods. We reported the relative rate constants for the DNPH derivatization reactions of formaldehyde, acetone, and acetaldehyde, by measuring the DNPH filter spiked with the sample.¹⁵ Using this previously reported reaction rate method¹⁵ is easier than preparing and measuring a sample of accurate concentration into air, but it requires the measurement of many samples to confirm the accuracy of the measurement. A simple method that can quickly determine the possibility of measuring other compounds using the existing method (for example, DNPH-formaldehyde method) is desired; however, no such simple method has yet been proposed or developed.

In this study, a simple test to check the order of reaction rates with DNPH was studied. Furthermore, the feasibility of this method was tested by spiking the mixture sample solution to the DNPH filter.

2 | EXPERIMENTAL METHODS

2.1 | Reagents and apparatus

Formaldehyde solution (37%) stabilized with methanol was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Acetone (99.8%, Environmental Analysis grade), acetonitrile (99.8%, Aldehyde Analysis grade for the extractions and High-Performance Liquid Chromatography grade for HPLC-PDA analysis), aldehydes (formaldehyde and acetaldehyde)-DNPH mixed standard solution (each 0.1 μg aldehyde/ μl acetonitrile), formaldehyde-DNPH and acetone-DNPH (0.1 mg/ml in acetonitrile) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Acetaldehyde ($\geq 99.5\%$, ACS reagent grade) was purchased from Sigma-Aldrich Japan (Tokyo, Japan). The membrane filter (Cosmonice Filter S, pore size: 0.45 μm , filter diameter: 4 mm) was purchased from Nacalai tesque,

Inc (Kyoto, Japan). The DNPH-aldehyde passive sampler (of the same lot number) was purchased from SKC Inc. (Eighty Four, USA). These DNPH-aldehyde passive samplers were removed from their sliding covers to be used as DNPH-impregnated filters (reactive tapes) before use. The whole DNPH-impregnated filter was cut into four equal pieces. These quarter-DNPH-impregnated filters were used for measurements.

All the water used in the experiment was purified with Direct-Q UV3 (Merck Millipore and Sigma-Aldrich, Japan Headquarters, Japan).

2.2 | Preparation of the extraction sample of the DNPH-impregnated filter spiked with samples

Formaldehyde, acetaldehyde, and acetone were mixed with a molar concentration proportion of 1:1:1. Each compound concentration of the mixed solution was 0.03–3 mol/20 μl (Total amount: 0.09–9 mol/20 μl). And 0.03–3 mol/20 μl of formaldehyde, acetaldehyde, and acetone solution were prepared. With the light off, the sample solutions and the DNPH-impregnated filters (quarter DNPH filter) were heated for 30 min at 25°C, using a heat block in test tubes that were sealed with polytetrafluoroethylene (PTFE)-lined screw caps. With the lights remaining off, these sample solutions (5 μl) were spiked with capillary micro pipets into the DNPH filters in the test tubes, and 0.75 ml of acetonitrile per quarter DNPH filter was added to each test tube (Reaction time (derivatization time): 0 min, which means immediately the sample on the filter was extracted.). These test tubes were then sealed with PTFE-lined screw caps, vortexed, and then ultrasonicated for 10 min. These solutions were used as HPLC samples following filtrations using membrane filters. The amount of DNPH was measured by the amount of DNPH-formaldehyde, which was spiked as formaldehyde (0–135 μmol) onto the DNPH filter to be derivatized. The amount of DNPH-formaldehyde detected was 4–5 μmol /filter (DNPH < total samples, DNPH = total DNPH-samples).

3 | RESULT AND DISCUSSION

Figure 1(A) shows the difference in the amount of each DNPH derivatization when the mixture solutions were spiked to the DNPH filter. When the total amounts of samples (formaldehyde + acetaldehyde + acetone) in the mixture solution was <3 μmol << DNPH (when DNPH < total samples, DNPH = total DNPH-samples), i.e., each sample amount in the mixture solution was

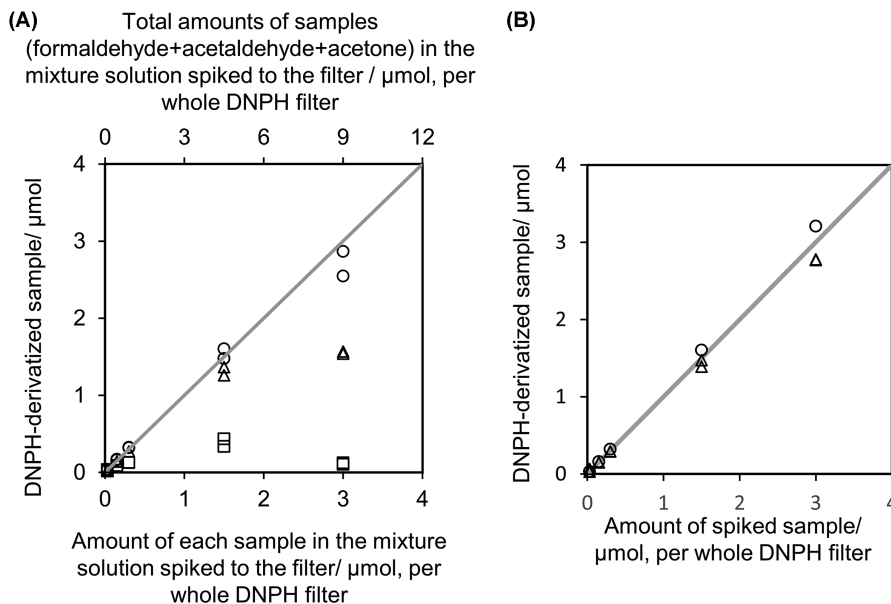


FIGURE 1 Difference of DNP-derivative amounts (the mixture solution(A), and the single sample solution(B)). (A) Sample: circles: formaldehyde; triangles: acetaldehyde; squares: acetone. Formaldehyde, acetaldehyde, and acetone amounts (μmol) in the mixture solution were 1:1:1. The mixture solutions were spiked to the DNP filter. Gray line: amount of DNP-derivative=amount of each spiked sample. (B) Sample: circles: formaldehyde solution spiked, triangles: acetaldehyde solution spiked. Gray line: amount of DNP-derivative = amount of spiked sample

<1 μmol (Figure 1(A)), there was little difference in the derivatization ratios among the compounds. However, when the amount of each sample in the mixture solution was 3 μmol , and the total amounts of samples (formaldehyde + acetaldehyde + acetone) \gg DNP, the derivatization ratios among the compounds were quite different. The order of the amounts of the DNP-derivatives was formaldehyde > acetaldehyde \gg acetone. However, the experimental conditions were such that the spiked molarity of formaldehyde, acetaldehyde, and acetone to the DNP filter were approximately the same, and DNP generally reacted with the aldehydes and the ketones. The yields of derivatization reactions were different, despite these conditions. This was due to the differences in the reaction rates. Acetone has been reported to react with DNP slower than formaldehyde.⁶ In a previous study, we estimated that the order of the relative reaction rate constants of formaldehyde, acetaldehyde, and acetone on the filter was formaldehyde > acetaldehyde \gg acetone.¹⁵ This order was the same as the order of the amounts of the DNP-derivatized samples when 3 μmol of each sample was spiked onto the filter. Furthermore, regarding the amounts of DNP-derivatives when the amount of DNP-formaldehyde was more than the amount of DNP-sample, the DNP reaction rate with formaldehyde > the DNP reaction rate with the other sample. This shows that when the amount of DNP-derivatives equaled the amount of spiked samples, all the spiked sample was derivatized with DNP. That is, when

the derivatization with DNP was a competitive reaction, and DNP < the total spiked samples that can react with DNP, the order of the derivatization amounts was the same as the order of the reaction rates. Furthermore, the difference in derivatization rates at DNP < total spiked samples was clearer than at DNP > total spiked samples. This was because all samples spiked with less than the DNP amounts could be derivatized, whereas a portion of the samples spiked with more than the DNP amounts could not be derivatized.

Figure 1(B) shows the amount of DNP-formaldehyde and DNP-acetaldehyde when formaldehyde solution and acetaldehyde solution were spiked to each DNP filter. All acetaldehyde and formaldehyde were derivatized with DNP, therefore no difference was found in the reaction rate of acetaldehyde and formaldehyde.

From these results, graphs were obtained by plotting the peak area, or the amount of substances of the DNP-derivatives that were extracted from the filter, on the Y-axis; the total amounts of samples spiked to the filter was plotted as the X-axis. These graphs can be used as a simple check test of the reaction rate comparison. They can also be used to check whether the DNP filter can be used for other samples. The check test method is as follows: in the first the sample mixture is prepared; it contains the same concentrations of each sample. The second step is obtaining the data, such as that displayed in Figure 1(A). The third step is checking the order of the amounts of the DNP-derivatives for DNP < the total amount of spiked

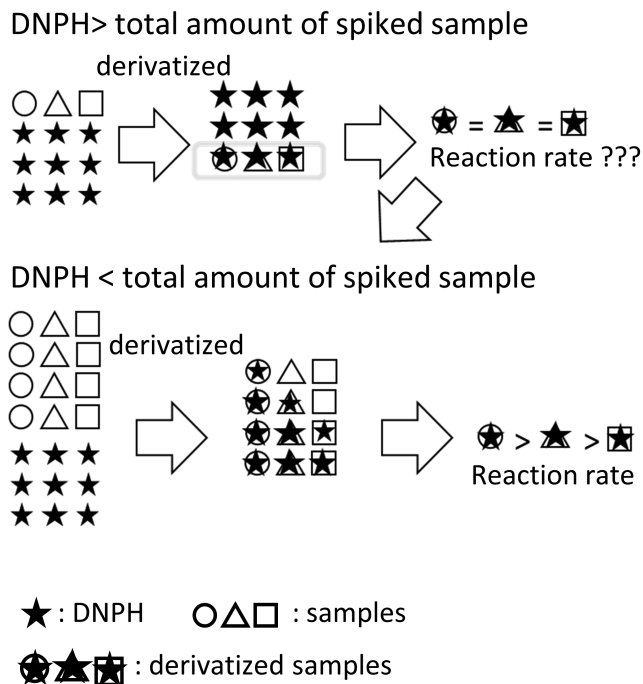


FIGURE 2 The third step of the simple test, and the relationship between reaction rate and derivatization amounts for the simple test confirmation

sample. If the amount of the DNPH-derivative is equal to the DNPH-other derivatives, a higher concentration sample mixture is spiked, the order of the DNPH-derivatives is checked (Figure 2). Through this method, the reaction rates of the samples were determined to be in the following order: formaldehyde > acetaldehyde >> acetone, and acetone did not react at the sampling rate of formaldehyde. In addition, this screening method can also visually clarify the order of reaction rates for samples with little difference in reaction rates, such as acetaldehyde and formaldehyde (Figure 1). If there is no difference in the amount of derivatization when the highest concentration sample were added, it will be necessary to be developed a reaction rate measuring device that can control the conditions with high accuracy.

4 | CONCLUSIONS

To develop a check test, sample mixtures containing the same concentrations of formaldehyde, acetaldehyde, and acetone were spiked to a DNPH-filter. They were then extracted, measured by HPLC-PDA, and the amounts of DNPH-derivatives versus the total amounts of the spiked samples were plotted.

This revealed that the amounts of DNPH-acetaldehyde and DNPH-acetone were not the same as that of DNPH-formaldehyde, and the order of the reaction rates was formaldehyde > acetaldehyde >> acetone.

These results showed that this check test can be confirmed that acetone is not collected at the sampling rate of formaldehyde. This check test (using the graph (Figure 1(A))) enables the easy measurement of the orders of reaction rates and the reactivity of other samples with DNPH (sampler). Furthermore, this check test enables the easy measurement of the slightly difference reaction rate order between samples. The test can also confirm, such as acetone measurement with DNPH sampler for formaldehyde, whether other samples can be measured by the analytical methods for the target sample.

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DISCLOSURE

Approval of the research protocol: N/A. Informed consent: N/A. Registry and the registration no. of the study/trial: N/A. Animal studies: N/A. Conflict of Interest: N/A.

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REFERENCES

- International Agency for Research on Cancer (IARC) FORMALDEHYDE. International Agency for Research on Cancer (IARC); [cited 2019 March 18]; Available from: <https://monographs.iarc.fr/wp-content/uploads/2018/06/mono100F-29.pdf>
- Occupational Safety and Health Administration OSHA Occupational Chemical Database. [cited 2019 March 18]; Available from: <https://www.osha.gov/chemicaldata/>
- Ministry of Health Labor, and Welfare Ministry of Health, Labor and Welfare laws and regulations database service (Japanese). [cited 2019 March 18]; Available from: <https://www.mhlw.go.jp/hourei/>
- Kozziel JA, Noah J, Pawliszyn J. Field sampling and determination of formaldehyde in indoor air with solid-phase microextraction and on-fiber derivatization. *Environ Sci Technol*. 2001;35:1481-1486.
- Uchiyama S, Inaba Y, Kunugita N. Derivatization of carbonyl compounds with 2,4-dinitrophenylhydrazine and their subsequent determination by high-performance liquid chromatography. *J Chromatogr B*. 2011;879:1282-1289.
- Ho SSH, Chow JC, Watson JG, et al. Biases in ketone measurements using DNPH-coated solid sorbent cartridges. *Anal Methods*. 2014;6(4):967-974. Available from: <https://doi.org/10.1039/C3AY41636D>
- Bicking MKL, Marcus Cooke W, Kawahara FK, Longbottom JE. Effect of pH on the reaction of 2,4-dinitrophenylhydrazine with formaldehyde and acetaldehyde. *J Chromatogr A*. 1988;455:310-315.
- Nakatani K, Sawada N, Sato T. Kinetic analysis of the solvent extraction of cadmium(II) complexes using a cadmium-deposited

- recessed microelectrode and confocal fluorescence microscope. *Anal Sci.* 2017;33:1081-1084.
9. Kohri S, Fujii H, Oowada S, et al. An oxygen radical absorbance capacity-like assay that directly quantifies the antioxidant's scavenging capacity against AAPH-derived free radicals. *Anal Biochem.* 2009;386:167-171.
 10. Komatsu-Watanabe R, Sakurai Y, Morimoto C, Sakamoto S, Kanaori K, Tajima K. Quantitative spin-trapping ESR investigation on reaction of hydroxyl radical and selected scavengers by a newly developed flow-injection ESR system. *Chem Lett.* 2008;37(6):612-613. Available from: <https://doi.org/10.1246/cl.2008.612>
 11. Takahashi T, Ohtsuka K-I, Iki N, Hoshino H. A microchip capillary electrophoretic reactor: a new methodology for direct measurement of dissociation kinetics of metal complexes. *Analyst.* 2005;130:1337-1339.
 12. Martos PA, Pawliszyn J. Sampling and determination of formaldehyde using solid-phase microextraction with on-fiber derivatization. *Anal Chem.* 1998;70:2311-2320.
 13. Finkelstein E, Rosen GM, Rauckman EJ. Spin trapping. Kinetics of the reaction of superoxide and hydroxyl radicals with nitrones. *J Am Chem Soc.* 1980;102:4994-4999.
 14. Sawada Y, Yamazaki I. One-electron transfer reactions in biochemical systems. VIII. Kinetic study of superoxide dismutase. *Biochimica Et Biophysica Acta (BBA) - Enzymology.* 1973;327(2):257-265. Available from: [https://doi.org/10.1016/0005-2744\(73\)90408-7](https://doi.org/10.1016/0005-2744(73)90408-7)
 15. Inoue N, Takaya M. Reactivity and relative reaction rates of formaldehyde, acetaldehyde, and acetone coexisting with large quantities of acetone on 2,4-dinitrophenylhydrazine-impregnated filters. *Anal Methods.* 2019;11:2785-2789. Available from: <https://doi.org/10.1039/C9AY00757A>

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