ISSN: 2085-675X (Print)
ISSN: 2354-8770 (Electronic)

Jurnal Kefarmasian Indonesia
Available online at https://jkefarind.com/index.php/jki
Original Research Article
JKI

The Effect of Temperature on Formaldehyde Migration and the Validation of Analytical Methods Used in Herbal Plastic Packaging

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ARTICLE INFO

Article history:
Received 10 June 2023
Revised 17 July 2023
Accepted 24 July 2023
Published online 31 August 2023

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DOI: https://doi.org/10.22435/jki.v13i2.6611

Citation: Nisma F, Riyanti HB, Julanda D. The Effect of Temperature on Formaldehyde Migration and the Validation of Analytical Methods Used in Herbal Plastic Packaging. Jurnal Kefarmasian Indonesia. 2023;13(2):175-183.

ABSTRACT

Plastic as food packaging has been the subject of extensive research, but plastic as packaging for herbs does not yet exist. As an additive, plastic made from the monomer and urea-formaldehyde is utilized. At elevated temperatures, formaldehyde can decompose due to the degradation of the monomer, followed by oxidation and the severing of the carbon chain. Formaldehyde can migrate to the bundled material, which is hazardous to human health. This study aimed to ascertain the impact of temperature on the migration of formaldehyde from plastic used to wrap herbs. The sample is a plastic bag used to wrap herbs, and NIR (Near Infrared) is used to determine the variety of plastic. Formaldehyde migration was determined by heating the sample between 40 and 80 degrees Celsius, and formaldehyde was analyzed using the UV-Vis spectrophotometer method with Nash reagent. The absorbance of formaldehyde was measured with a spectrophotometer at a wavelength of 412 nm. The type of plastic obtained was PP (Poly Propylene). The equation for the formaldehyde calibration curve is y = 0.0197x + 0.1218 with a correlation coefficient (r) of 0.999. The migration of formaldehyde was measured after heating plastic to temperatures between 40 and 80 degrees Celsius. The released formaldehyde concentrations ranged from 7.35 to 13.47 µg/mL. Validation of the analytical method revealed the formaldehyde detection limit (LOD) to be 0.8024 µg/mL and the quantity limit (LOQ) to be 2.6745 µg/mL, with a precision of 1 and an accuracy of 97,462-113,851%, thereby satisfying the meticulous, exhaustive, and precise criteria.

Keywords: Formaldehyde migration; Temperature variation; Plastic herbal packaging; Method validation

INTRODUCTION

Herbal and beverage containers are commonly made of plastic, both in industry and at home. Plastics are flexible, durable, inexpensive, readily available, and resistant to microbial contamination. Some plastics are flexible while others are rigid; flexible plastics are utilized more frequently than rigid plastics. 53% of the plastic used for packaging, preserving, and wrapping herbs is flexible, whereas beverages are commonly packaged in rigid containers.[1]

Plastic also has the advantages of being light, rust-resistant, inert, thermoplastic (easily formed with heat), and colorable, in addition to being strong and flexible. Ethane and propane, the primary components of plastic, are derived from petroleum and broken down at high temperatures into ethylene and propylene.[2] As plasticizers, fillers, and dyes, or to enhance the physicochemical properties, various additives such as glycerol, isopropyl alcohol, urea, and formaldehyde are added along with a catalyst to form plastic polymers, which are then formed

ISSN: 2085-675X (Print)
ISSN: 2354-8770 (Electronic)

into plastic pellets or ores. According to their designation, plastic seedlings undergo additional processing to become plastic products.[3]

Plastics are produced by polymerizing ethylene or propylene monomers and adding additives to preserve and enhance their physicochemical properties. The additive agent is comprised of plasticizers, pigments, glycerol, isopropyl alcohol, urea, and formaldehyde. In the plastics and adhesives industries, formaldehyde is utilized to create urea-formaldehyde resins. Additionally, formaldehyde can be added to plastic to make it shiny, brilliant, or transparent.[4,5]

Herbal packaging is typically made of transparent or translucent plastic. These plastics are only recommended for one-time use at room temperature because it is anticipated that repeated use, particularly for hot herbs or food, could cause the polymer layer to degrade and release potentially harmful substances. From the plastic, antimony trioxide compounds and formaldehyde can be released. Moreover, formaldehyde can be released from plastic by light, heat, and ozone.[6]

Formaldehyde that has been released can migrate to packaged herbs. This migration diminishes the quality and safety of herbs. Toxic monomers and formaldehyde can rapidly migrate into liquid, viscous, and warm herbs. The higher the temperature of the herb, the greater the expected migration of formaldehyde. The amount of these compounds that migrate is insignificant enough to go unnoticed but can be fatal over time. The likelihood of toxic formaldehyde migration increases with contact time, temperature, formaldehyde concentration in the plastic, and the reactivity of herbal constituents.[7]

Formaldehyde compounds can be converted to formic acid in the human body, which can result in acidosis (leading to shortness of breath), liver dysfunction, cancer, hypothermia, coma, and even mortality. If formaldehyde comes into contact with the eyes, it can cause irritation and visual disturbances. If ingested, it can cause irritation of the pharynx.[7]

Formaldehyde can migrate from melamine tableware receptacles heated between 60 and 80 degrees Celsius. The same phenomenon can occur in plastics that contain formaldehyde or formaldehyde-urea. Alotaibi et al. reported that melamine used in household appliances contains significant levels of formaldehyde.[8] Prior research on the formaldehyde content of various plastic herbal and beverage containers revealed a relatively high formaldehyde level in some of the herbal containers examined.

In light of the preceding, it is necessary to analyze the amount of formaldehyde migration in plastic botanical packaging heated between 40°C and 80°C. After adding the Nash reagent, a spectrophotometric method was used to analyze formaldehyde. The concentration of formaldehyde can be calculated from the absorbance of the yellow solution as measured by a UV-vis spectrophotometer after the Nash reagent reacts with formaldehyde to form a yellow product.[9]

We then determined the levels of formaldehyde that migrated at different temperatures of immersion in order to assess the suitability of the analytical method. By assessing linearity, precision, accuracy, LOD, and LOQ, the analytical method's linearity, precision, and accuracy were validated. Standard deviation or relative standard deviation was used to quantify precision. Precision is equivalent to repeatability or reproducibility. Accuracy is the similarity between the determined results and the actual results. Recovery of the added analyte is an expression of precision.[10] In a given concentration range, linearity is the capacity of a method to produce test results that are directly proportional to the analyte concentration. The calibration curve relates the response (y) to the concentration (x) is a method's linearity.

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ISSN: 2354-8770 (Electronic)

It was possible to measure linearity with a single measurement at various concentrations. LOD is the lowest concentration of analyte in a sample that can still be detected, whereas LOQ is the lowest concentration of analyte that can be determined with acceptable precision and accuracy under the operational conditions of the method being employed.[11]

Research on plastic as food packaging has been widely studied but plastic as herbal packaging does not exist, even though many herbs are wrapped in plastic and do not have proper handling, like storing herbs at unsuitable temperatures. The purpose of this research is to determine the effect of temperature on formaldehyde migration and the validation of analytical methods used in herbal plastic packaging.

METHODS

Materials and methods

UV-vis spectrophotometer (Shimadzu), NIR spectrophotometer (Phazir), Analytical Balance, Hot plate, micropipette, and thermometer were utilized. All chemicals utilized were of analytical grade and procured from Brand: formaldehyde (Merck, 37%), acetylacetone (Merck), glacial acetic acid (Merck, 100%), ammonium acetate (Merck), phosphoric acid (Merck, 75%), hydrochloric acid (Merck), aqua dest, plastic wrapping.

Sample

The sample was a plastic bag commonly used to swaddle food or herbs and sold for free. Two distinct varieties of colorless, transparent, rigid, sturdy, and bright 1530 cm plastic bags were utilized. PP (polypropylene) plastic makes up the sample's defining characteristic.[12]

Qualitative analysis

The qualitative analysis test was conducted by undertaking a formaldehyde color test with Nash reagent. Previously, 10 mL of distilled water was placed in a plastic container and heated for 10 minutes. 1 mL of water was extracted from the container and combined with 5 mL of Nash reagent.

Determination of the maximum wavelength of formaldehyde using a UV-vis spectrophotometer

1 mL of formaldehyde was pipetted from the standard solution into an Erlenmeyer flask containing 5 mL of Nash reagent and 2.5 mL of distilled water. The Erlenmeyer flask was then wrapped in aluminum foil and agitated until the solution was uniform. The solution was heated for 30 minutes at 37°C in a water boiler. After chilling, the solution was transferred to a 25 mL volumetric flask, the volume was adjusted with distilled water, and the flask was then shaken until uniform. Using a UV-vis spectrophotometer, absorbance was measured between 300 and 500 nm to ascertain the wavelength of maximum absorbance.

Time optimization (operating time)

The standard solution of formaldehyde was pipetted into an Erlenmeyer flask. Add 5 mL of the Nash reagent and 10 mL of distilled water. The Erlenmeyer flask was wrapped in aluminum foil, and the solution was agitated until uniform. The solution was heated for 30 minutes at 37oC in a water boiler. After chilling, the solution was transferred to a 25 mL volumetric flask, the volume was adjusted with distilled water, and the flask was shaken until the solution was homogenous. The solution was measured with a UV-vis spectrophotometer for 30 minutes at the maximal wavelength previously determined. Changes in absorbance over time were used to ascertain stability.

Quantitative analysis

Collecting water from heated plastic containers, adding Nash reagent, and measuring absorbance at the maximum wavelength on a spectrophotometer. For each experimental unit, all procedures were repeated five times.

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Determination of migration levels of formaldehyde at a certain temperature of heating.

Each plastic container contained 100 mL of distilled water that was heated to 40°, 50°, 60°, 70°, 80°, and 90°C. In an Erlenmeyer flask, 1 mL of each solution was heated to various temperatures of 40°, 50°, 60°, 70°, and 80°C. Also added were 5 mL of Nash reagent and 10 mL of distilled water. The absorbance of the solution was then measured at its maximal wavelength using a UV-vis spectrophotometer after the solution was thoroughly mixed.

Method Validation

Linearity

From a solution of 250 µg/mL, a concentration series of formaldehyde at 7, 12, 17, 21, and 26 µg/mL was prepared. A total of 0.7 mL of the solution was added to a 25 mL volumetric vial, along with 1.2 mL, 1.7 mL, 2.1 mL, and 2.6 mL of distilled water to bring the volume to 25 mL. 1 mL of each solution was pipetted into a test tube. Five milliliters of Nash reagent were added, dissolved in distilled water, and then covered with aluminum foil. The solution was heated for 30 minutes at 37oC in a water boiler. After cooling, the absorbance was measured using a UV-vis spectrophotometer at the maximal wavelength. The data were fitted with a linear equation of the form y = bx+a, and the correlation coefficient (r) was calculated.

LOD dan LOQ

Limits of Detection (LOD) and Quantitation (LOQ) are determined. LOD and LOQ are computed using the linear equation of the calibration curve and the following formula:

$$Q = \frac{k\,x\,S}{s1}$$

where Q is the LOD or LOQ, k is 3.3 for the detection limit or 10 for the quantitation limit, S is the standard deviation of the analytical response and blank, and s1 is the slope of the linear relationship between response and concentration.

Limits of detection and quantification can be calculated statistically using the calibration curve's linear regression line. The measurement value would equal the value of b in the linear equation y= a+bx, whereas the null standard deviation would equal the residual standard deviation (Sy/x).

The values of the relative standard deviation (S) and coefficient of variation (CV) were calculated using the following equations:

$$s = \sqrt{\frac{\sum_{i=1}^{n}\left(x_i - \bar{x}\right)^2}{n-1}}$$

$$\%\ CV = \frac{S\ X\ 100\%}{x}$$

Where X1 is the value of a single measurement, X is the average, and n is the number of measurements.[11]

Accuracy.

In a water immersion, 100 mL of the test sample was reduced to 30 mL in a glass beaker containing 30 mL of water. The mixture was then transferred to a 50 mL volumetric flask and topped off with 50 mL of distilled water. Two milliliters of the test sample were added to a 10-milliliter volumetric vial. 1 mL of standard intermediate (25 µg/mL) and 5 mL of Nash reagent were added to a volume of 10 mL of distilled water, and the mixture was agitated until uniform. The absorbance was then determined using a UV-vis spectrophotometer after a 5-minute incubation and further shaking. The recovery value was calculated by dividing the concentration obtained from the formaldehyde product by the actual concentration of formaldehyde and multiplying the result by 100 to obtain a percentage.[11]

$$\text{Recovery} = \frac{concentration\ obtained}{real\ concentration}\ x\ 100\%$$

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Quantitative analysis of samples

1 mL of heated samples at 40°, 50°, 60°, 70°, and 80°C were added to an erlenmeyer flask along with 5 mL of Nash reagent and 10 mL of distilled water. At the maximal wavelength, the absorbance of the solution was measured using a UV-vis spectrophotometer after it was thoroughly mixed. Using an equation for linear regression, the formaldehyde content of the samples was determined:

$$y = a + bx$$

where y is the dependent variable (absorbance), a is the intercept (the point at which the curve intersects the y-axis), b is the slope (slope), and x is the formaldehyde concentration.

RESULTS AND DISCUSSION

In this investigation, samples of plastic bags used to wrap over-the-counter herbal products were utilized. These plastics are commonly employed for wrapping heated herbs. Purposive sampling was used for the sampling, in which population characteristics or previously known characteristics were taken into account.[13] Figure 1 depicts the samples utilized in the investigation.

Figure 1. Plastic test sample

Plastic type analysis

The type of plastic was identified using infrared (NIR) devices.

Table 1. The results of the analysis of the type of plastic test samples

Sample	Sample Type
Pouch 1	PP
Pouch 2	PP

Both samples were determined to be polypropylene (PP). Clear, transparent, inelastic, hygienic, clean, and odorless PP is widely available as plastic bags, coils, and sheets. PP plastic is resistant to acids and bases, but hydrocarbons and oxidizing agents readily degrade it. PP is also combustible, brittle below 20°C, and begins to lose its structural integrity at 120°C. UV exposure causes PP to become brittle, so it is advised that this packaging be used only once.[2]

Qualitative Analysis

Qualitative tests of the formaldehyde content in the herbal packaging plastic samples involved filling the plastic samples with water and heating them to different temperatures. The formaldehyde content was analyzed by adding Nash solution to form a yellow color.

Table 1. Qualitative analysis of formaldehyde migration from plastic packaging

Temperature (°C)	Sample 1	Sample 2
40	+	+
50	++	++
60	+++	+++
70	++++	++++
80	+++++	+++++

Note: The + sign indicates the strength of the yellow color in the solution.

Table 1 displays the results of the qualitative analysis performed. Each and every test solution contained formaldehyde. The greater the heating temperature, the greater the formaldehyde concentration. Due to the condensation reaction of the plantation -(2,4) (acetylacetone) with formaldehyde and ammonia to produce 3,5-diethyl-2,6-dimethyl-1,4-dihydropyridine, the formaldehyde solution turned yellow when the Nash reagent was added. The yellow hue is the result of the construction of a stable and reversible monomer system.[9]

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Formaldehyde maximum wavelength

According to the Indonesian Pharmacopeia, Edition IV, the maximal absorbance of formaldehyde is 415 nm.14 The peak absorbance of our samples occurred at 412.00 nm. Consequently, our measurement results satisfied the criteria for use in the analysis.

Determination of calibration curve

Standard solutions were prepared using formaldehyde with concentrations of 7, 12, 17, 21, and 26 µg/mL, and measuring absorbance after the addition of Nash reagent, a calibration curve was constructed. Figure 3 demonstrates the calibration curve.

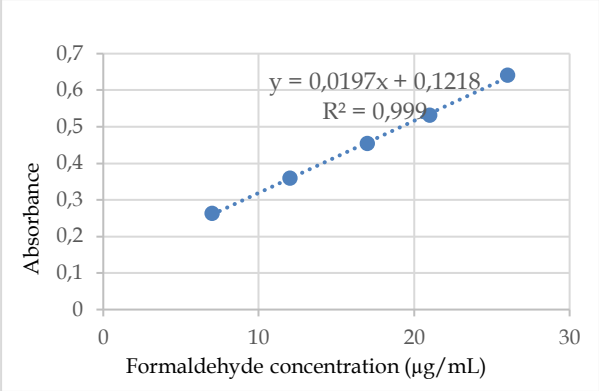

Figure 2. Standard of formaldehyde calibration curve

The correlation coefficient (r) for the calibration curve was 0.999. The equation for the calibration curve was y = 0.0197x + 0.1218. The increase in the analyte absorption value was proportional to the formaldehyde concentration. The correlation coefficient had to be at least 0.999 (15), per the criteria and conditions. The correlation coefficient indicates that the assay is linear over the examined range.

Determination of the migration of formaldehyde as a function of temperature

The results of the study of the migration of formaldehyde from plastic bags heated to 40–90°C are shown in Table 2.

Table 2. The effect of temperature on the migration of formaldehyde

Temperature (°C)	Absorbance	Migration of Formaldehyde (µg/mL)	Average (µg/mL)
40	0.2670	7.37	7.35
	0.2651	7.27	
	0.2658	7.31	
	0.2649	7.26	
	0.2679	7.42	
	0.2643	7.42	
50	0.2828	8.17	8.15
	0.2866	8.36	
	0.2886	8.47	
	0.2778	7.92	
	0.2822	8.14	
	0.2769	8.87	
60	0.2997	9.03	9.05
	0.2973	8.91	
	0.2975	8.92	
	0.3038	9.47	
	0.2972	8.90	
	0.2990	8.99	
70	0.3053	9.22	9.31
	0.3051	9.30	
	0.3057	9.33	
	0.3058	9.34	
	0.3057	9.33	
	0.3053	9.32	
80	0.3761	12.91	12.37
	0.3711	12.65	
	0.3710	12.65	
	0.3714	12.68	
	0.2572	11.65	
	0.3464	11.40	

Plastic is generally regarded as an inert substance. However, if there is friction on the plastic's surface and the temperature rises, chemical bonds can break on the plastic's surface, releasing the monomers and additives used to make the plastic. Thus, formaldehyde can be released and migrated to packaged herbs.[8] Decomposition and migration can occur rapidly if the temperature of the herb is elevated. Formaldehyde levels in bottled potable water in Makassar, South Sulawesi, were as high as 16.45 µg/mL, according to a study on formaldehyde migration.[6]

The formaldehyde content increased as the plastic was heated. This suggests that hot herbals, such as meatballs, hot soup, or

ISSN: 2085-675X (Print)
ISSN: 2354-8770 (Electronic)

rice, should not be packaged in plastic bags, as high-temperature herbals (> 60 °C) can cause the migration of formaldehyde from packaging to the herbal or food, which could be harmful to the health of those who ingest it.

Formaldehyde has a flashpoint of 64 degrees Celsius, above which it is intensely reactive. This indicates that precautions must be taken to avoid wrapping herbs in plastic above this temperature.[16]

LOD and LOQ

The detection limit is the smallest amount of analyte that can be detected and still give a significant response. The quantitation limit is a parameter in the analysis that acts as the smallest amount of analyte in the sample that can still meet the criteria carefully and thoroughly. Limits of detection and quantitation are calculated from a standard curve. Based on Figure 3, the regression line equation is y = 0.0197x + 0.1218. LOD = 3,3 (SD/S) dan LOQ = 10 (SD/S) (SD = Deviation standart and S = slope of the regression equation).(17) The detection limit (LOD) of formaldehyde was determined to be 0.8024 µg/mL and the LOQ value obtained was 2.6745 µg/mL

Salman Alotaibi et al. (2021) conducted a study on the determination of migrated formaldehyde from kitchenware using gas chromatography-mass spectrometry; the results obtained for the LOD and LOD values of the respective methods were 0.05 and 0.142 µg/mL; the concentrations obtained were lower as a result of the different analytical methods; and the GC analysis method was more sensitive than a spectrophotometer.[5]

Table 3. The Value of LOD and LOQ

Item	Value
r	0,999
SD	0,00527
LOD (µg/mL)	0.8024
LOQ (µg/mL)	2.6745

Accuracy

Accuracy is the closeness of the analysis results to the actual analyte content. Table 4 shows the results of the accuracy determinations using a standard solution of 10 µg/mL heated to temperatures of 40–80°C.

Table 4. Accuracy analysis results

Temperature (°C)	Replication	Absorbent	Recovery (%)
40	1	0,3164	98,782
	2	0,3149	98,020
	3	0,3138	97,462
	4	0,3166	98,883
	5	0,3146	97,868
	6	0,3160	98,579
50	1	0,3259	103,50
	2	0,3273	104,00
	3	0,3257	103,35
	4	0,3261	103,71
	5	0,3275	104,42
	6	0,3262	103,76
60	1	0,3302	103,50
	2	0,3305	104,00
	3	0,3312	103,35
	4	0,3307	103,71
	5	0,3302	104,42
	6	0,3306	103,76
70	1	0,3319	106,65
	2	0,3317	106,55
	3	0,3324	106,41
	4	0,3318	106,51
	5	0,3328	107,11
	6	0,3324	106,90
80	1	0,3439	112,74
	2	0,3435	112,54
	3	0,3459	113,76
	4	0,3455	113,55
	5	0,3473	109,39
	6	0,3460	113,81
Average recovery (%)			105,391

Accuracy is achieved with an average formaldehyde recovery of 105.391% and a range of 97.462-113.81% for temperatures between 40 and 80 degrees Celsius. The acceptable percent recovery requirements range from 97 to 103%. In Alotaibi's study

ISSN: 2085-675X (Print)
ISSN: 2354-8770 (Electronic)

on the determination of migrated formaldehyde from kitchenware using gas chromatography-mass spectrometry, the recovery value was found to be between 97.64 and 99.43%; this research is not significantly different.5 So the accuracy results are only acquired by heating the plastic sample to 60 degrees Celsius.

Precision

Precision or accuracy is the ability to obtain an average value that is extremely near to the true value and is determined by calculating the standard deviation (SD) and coefficient of variation. These values can be found in Table 5.

Table 5. Calculation of precision at each temperature

Sample temperature (^{0}C)	S (%)	RS (%)
40	0.07	0.89
50	0.24	2.89
60	0.22	2.40
70	0.04	0.47
80	0.58	4.66

The precision values obtained were all < 1, meaning that the concentration of formaldehyde can be determined precisely to within 1 µg/mL, indicating acceptable reproducibility. The measurement results obtained were accurate because they were close to the actual values. Our data satisfies the requirement that the precision is < 5% (18). Accuracies in the form of recovery values are tabulated in Table 4. At all temperatures, the recovery value was below 120% and met the requirement that the recovery value should be between 97,462-113,851%, which could be observed in fulfilling the careful, thorough, and accurate criteria.[14]

In Alotaibi's study, the recovery value ranged from 97.64 to 99.43%, whereas Hayun et al.'s (2017) study on the determination of formaldehyde content in wet noodles by thin-layer chromatography densitometry after derivatization with Nash reagent yielded a recovery value of 94.17 %, which is comparable to the results of the present study.

CONCLUSION

According to the findings of the present investigation, the plastic used for packaging herbs is PP (polypropylene). Temperature affected formaldehyde migration from packaging bags; the higher the temperature, the more formaldehyde is released from plastic herb packaging. Therefore, care should be taken when using plastic containers for hot dishes or herbs. Valid, accurate, and exhaustive, the UV-Vis spectrophotometer validation method used to determine the level of formaldehyde migration from plastic bags containing herbs can be accepted as an acceptable validation method.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgment

The author thanks the UHAMKA research institute has funded this research.

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