

Formic acid

UV-method

for the determination of formic acid in foodstuffs and other materials

Cat. No. 10 979 732 035

Test-Combination for 21 determinations

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Enzymatic BioAnalysis / Food Analysis

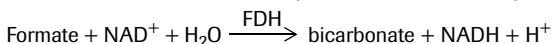
For *in vitro* use only

Store at 2-8°C

For recommendations for methods and standardized procedures see references (2)

Principle (Lit. 1)

Formic acid (formate) is quantitatively oxidized to bicarbonate by nicotinamide-adenine dinucleotide (NAD) in the presence of formate dehydrogenase (FDH).



The amount of NADH formed is stoichiometric to the amount of formic acid. The increase in NADH is measured by means of its light absorbance at 334, 340 or 365 nm.

The Test-Combination contains

1. Bottle 1 with approx. 22 ml solution, consisting of:
potassium phosphate buffer, pH approx. 7.5
2. Bottle 2 with approx. 420 mg NAD, Li salt, lyophilizate
3. Bottle 3 with formate dehydrogenase lyophilizate, approx. 80 U

Preparation of solutions

1. Dissolve contents of bottle 2 with the contents of bottle 1 using a magnetic stirrer, if necessary (= reaction mixture 2).
2. Dissolve contents of bottle 3 with 1.2 ml redist. water (= solution 3).

Stability of reagents

Solution 1 is stable at 2-8°C (see pack label).

Bring solution 1 to 20-25°C before use.

The contents of bottle 2 are stable at 2-8°C (see pack label).

Reaction mixture 2 is stable for 2 weeks at 2-8°C.

Bring reaction mixture 2 to 20-25°C before use.

The contents of bottle 3 are stable at 2-8°C (see pack label).

Solution 3 is stable for 5 days at 2-8°C, for 3 weeks at -15 to -25°C.

Procedure

- Wavelength¹: 340 nm, Hg 365 nm or Hg 334 nm
Glass cuvette²: 1.00 cm light path
Temperature: 20-25°C
Final volume: 3.050 ml
Read against air (without a cuvette in the light path) or against water or against blank³
Sample solution: 0.4-20 µg formic acid/assay⁴ (in 0.100-2.000 ml sample volume)

Pipette into cuvettes	Blank	Sample
reaction mixture 2	1.000 ml	1.000 ml
sample solution*	-	0.100 ml
redist. water	2.000 ml	1.900 ml
Mix**, read absorbances of the solutions (A ₁) after approx. 5 min. Start reaction by addition of:		
solution 3	0.050 ml	0.050 ml
Mix**, close the cuvettes*** and wait for the end of the reaction (20 min at 20-25°C). Read absorbances of blank and sample immediately one after another (A ₂).		

* Rinse the enzyme pipette or the pipette tip of the piston pipette with sample solution before dispensing the sample solution.

** For example, with a plastic spatula or by gentle swirling after closing the cuvette with Parafilm (trademark of the American Can Company, Greenwich, Ct., USA)

*** For example, with Parafilm (trademark of the American Can Company, Greenwich, Ct., USA)

Determine the absorbance differences (A₂-A₁) for both, blank and sample. Subtract the absorbance difference of the blank from the absorbance difference of the sample.

$$\Delta A = (A_2 - A_1)_{\text{sample}} - (A_2 - A_1)_{\text{blank}}$$

1 The absorption maximum of NADH is at 340 nm. On spectrophotometers, measurements are taken at the absorption maximum; if spectralline photometers equipped with a mercury vapor lamp are used, measurements are taken at a wavelength of 365 nm or 334 nm.

2 If desired, disposable cuvettes may be used instead of glass cuvettes.

3 For example by using a double-beam photometer

4 See instructions for performance of assay

The measured absorbance differences should, as a rule, be at least 0.100 absorbance units to achieve sufficiently precise results (see "Instructions for performance of assay" and "Sensitivity and detection limit", pt. 4).

Calculation

According to the general equation for calculating the concentration:

$$c = \frac{V \times MW}{\varepsilon \times d \times v \times 1000} \times \Delta A \text{ [g/l]}$$

V = final volume [ml]

v = sample volume [ml]

MW = molecular weight of the substance to be assayed [g/mol]

d = light path [cm]

ε = extinction coefficient of NADH at:

$$340 \text{ nm} = 6.3 \text{ [l} \times \text{mmol}^{-1} \times \text{cm}^{-1}\text{]}$$

$$\text{Hg } 365 \text{ nm} = 3.4 \text{ [l} \times \text{mmol}^{-1} \times \text{cm}^{-1}\text{]}$$

$$\text{Hg } 334 \text{ nm} = 6.18 \text{ [l} \times \text{mmol}^{-1} \times \text{cm}^{-1}\text{]}$$

It follows for formic acid:

$$c = \frac{3.050 \times 46.03}{\varepsilon \times 1.00 \times 0.100 \times 1000} \times \Delta A = \frac{1.404}{\varepsilon} \times \Delta A \text{ [g formic acid/l sample solution]}$$

If the sample has been diluted on preparation, the result must be multiplied by the dilution factor F.

When analyzing solid and semi-solid samples which are weighed out for sample preparation, the result is to be calculated from the amount weighed:

$$\text{Content}_{\text{formic acid}} = \frac{c_{\text{formic acid}} \text{ [g/l sample solution]}}{\text{weight}_{\text{sample}} \text{ in g/l sample solution}} \times 100 \text{ [g/100 g]}$$

1. Instructions for performance of assay

The amount of formic acid present in the assay has to be between 1 µg and 20 µg (measurement at 365 nm) or 0.4 µg and 10 µg (measurement at 340, 334 nm), respectively. In order to obtain a sufficient absorbance difference, the sample solution is diluted to yield a formic acid concentration between 0.04 and 0.2 g/l or 0.02 and 0.1 g/l, respectively.

Dilution table

Estimated amount of formic acid per liter		Dilution with water	Dilution factor F
measurement at			
340 or 334 nm	365 nm		
< 0.1 g	< 0.2 g	-	1
0.1-1.0 g	0.2-2.0 g	1 + 9	10
1.0-10 g	2.0-20 g	1 + 99	100
> 10 g	> 20 g	1 + 999	1000

If the measured absorbance difference (ΔA) is too low (e.g. <0.100), the sample solution should be prepared again (weigh out more sample or dilute less strongly) or the sample volume to be pipetted into the cuvette can be increased up to 2.000 ml. The volume of water added must then be reduced to obtain the same final volume in the assays for sample and blank. The new sample volume v must be taken into account in the calculation.

2. Technical information

2.1 Materials for decalcification may contain formic acid. Therefore, glass ware used for production and storage of solutions for the determination of formic acid have to be rinsed carefully with tap water and with redist. water afterwards after decalcification. (Formic acid in the reagents is responsible for a high blank absorbance difference.)

2.2 The volatility of formic acid has to be taken into consideration when assay control solutions are produced. Therefore, formic acid is always pipetted under the surface of (weakly alkaline) solutions.

2.3 The reagents, especially NAD, have to be free of formic acid.

2.4 In carrying out the calculation, a clear indication should be given as to whether the results are to be given as formic acid (molar mass 46.03 g/mol) or as formate (molar mass 45.02 g/mol). (In enzymatic determinations, the formate ion is measured.)

3. Specificity (Ref. 1)

The method is specific for formic acid. Acetic acid, propionic acid, oxalic acid and L-ascorbic acid do not influence the determination. Formaldehyde reduces the reaction rate but does not influence the specificity of the method.

In the analysis of commercial formic acid results of < 100% have to be expected because formic acid is split into CO and water during storage. (The volatility of formic acid has to be considered when formic acid solutions are produced.)

4. Sensitivity and detection limit (Ref. 1.2)

The smallest differentiating absorbance for the procedure is 0.004 absorbance units. This corresponds to a maximum sample volume $v = 2.000$ ml and measurement at 340 nm of a formic acid concentration of 0.05 mg/l sample solution (if $v = 0.100$ ml, this corresponds to 1 mg/l sample solution).

The detection limit of 0.2 mg/l is derived from the absorbance difference of 0.020 (as measured at 340 nm) and a maximum sample volume $v = 2.000$ ml.

5. Linearity

Linearity of the determination exists from approx. 0.4 μg formic acid/assay (0.2 mg formic acid/l sample solution; sample volume $v = 2.000$ ml) to 20 μg formic acid/assay (0.2 g formic acid/l sample solution; sample volume $v = 0.100$ ml).

6. Precision

In a double determination using one sample solution, a difference of 0.005 to 0.010 absorbance units may occur. With a sample volume of $v = 0.100$ ml and measurement at 340 nm, this corresponds to a formic acid concentration of approx. 1-2 mg/l. (If the sample is diluted during sample preparation, the result has to be multiplied by the dilution factor F. If the sample is weighed in for sample preparation, e.g. using 1 g sample/100 ml = 10 g/l, a difference of 0.01-0.02 g/100 g can be expected.)

The following data have been published in the literature:

CV = 1.0-2.2 % (Ref. 1.1)

Tomato pulp, tomato ketchup:

$r = 0.01$ g/100 g $s_{(r)} = \pm 0.004$ g/100 g
 $R = 0.02$ g/100 g $s_{(R)} = \pm 0.007$ g/100 g (Ref. 2.3)

CV = 0.65-1.64 % (formic acid solutions)

CV = 0.95-2.40 % (apple juices)

CV = 0.48-2.25 % (black currant nectars)

CV = 0.67-2.15 % (nectars from sour cherries) (Ref. 3.3)

7. Interference/sources of error

7.1 Reducing substances in the sample can diminish the reaction rate. In the presence of 10 μg sulfur dioxide in the assay the reaction stops after approx. 40 min. SO_2 can be destroyed by addition of 10 μl hydrogen peroxide (30%, w/v) to the assay.

L-Ascorbic acid does not interfere with the assay - even in high amounts.

7.2 In the presence of formaldehyde the formic acid reaction is inhibited. Small amounts of formaldehyde (5 μg /assay) diminish the reaction rate of the formic acid reaction. 10 μg formaldehyde give a small inhibition and 100 μg a strong inhibition of the enzyme FDH.

7.3 Interferences of the assay by amines (e.g. fish components) have not been observed.

8. Recognizing interference during the assay procedure

8.1 If the conversion of formic acid has been completed according to the time given under "Procedure", it can be concluded in general that no interference has occurred.

8.2 On completion of the reaction, the determination can be restarted by adding sodium formate (qualitative or quantitative): if the absorbance is altered subsequent to the addition of the standard material, this is also an indication that no interference has occurred.

8.3 Operator error or interference of the determination through the presence of substances contained in the sample can be recognized by carrying out a double determination using two different sample volumes (e.g. 0.100 ml and 0.200 ml): the measured differences in absorbance should be proportional to the sample volumes used.

When analyzing solid samples, it is recommended that different quantities (e.g. 1 g and 2 g) be weighed into 100 ml volumetric flasks. The absorbance differences measured and the weights of sample used should be proportional for identical sample volumes.

8.4 Possible interference caused by substances contained in the sample can be recognized by using an internal standard as a control: in addition to the sample, blank and standard determinations, a further determination should be carried out with sample **and** assay control solution in the same assay. The recovery can then be calculated from the absorbance differences measured.

8.5 Possible losses during the determination can be recognized by carrying out a recovery test: the sample should be prepared and analyzed with and without added standard material. The additive should be recovered quantitatively within the error range of the method.

9. Reagent hazard

The reagents used in the determination of formic acid are not hazardous materials in the sense of the Hazardous Substances Regulations, the Chemicals Law or EC Regulation 67/548/EEC and subsequent alteration, supplementation and adaptation guidelines. However, the general safety measures that apply to all chemical substances should be adhered to.

After use, the reagents can be disposed of with laboratory waste, but local regulations must always be observed. Packaging material can be disposed of in waste destined for recycling.

10. General information on sample preparation

In carrying out the assay:

Use **clear, colorless and practically neutral liquid samples** directly, or after dilution according to the dilution table, and of a volume up to 2.000 ml;

Filter **turbid solutions**;

Degas **samples containing carbon dioxide** (e.g. by filtration);

Adjust **acid samples** to pH 7-8 by adding sodium or potassium hydroxide solution;

Adjust **acid and weakly colored samples** to pH 7-8 by adding sodium or potassium hydroxide solution and incubate for approx. 15 min;

Measure **"colored" samples** (if necessary adjusted to pH 7-8) against a sample blank (= buffer or redist. water + sample), adjust the photometer to 0.000 with the blank in the beam;

Treat **"strongly colored" samples** that are used undiluted or with a higher sample volume with activated charcoal (e.g. 2 g/100 ml);

Crush or homogenize **solid or semi-solid samples**, extract with water or dissolve in water and filter if necessary; alternatively remove turbidities and dyestuffs with Carrez reagents;

Deproteinize **samples containing protein** with perchloric acid or with trichloroacetic acid; alternatively clarify with Carrez reagents.

Carrez clarification:

Pipette the liquid sample into a 100 ml volumetric flask which contains approx. 60 ml redist. water, or weigh sufficient quantity of the sample into a 100 ml volumetric flask and add approx. 60 ml redist. water. Subsequently, carefully add 5 ml Carrez-I-solution (potassium hexacyanoferrate(II) (ferrocyanide), 85 mM = 3.60 g $\text{K}_4[\text{Fe}(\text{CN})_6] \times 3 \text{H}_2\text{O}/100$ ml) and 5 ml Carrez-II-solution (zinc sulfate, 250 mM = 7.20 g $\text{ZnSO}_4 \times 7 \text{H}_2\text{O}/100$ ml). Adjust to pH 7.5-8.5 with sodium hydroxide (0.1 M; e.g. 10 ml). Mix after each addition. Fill the volumetric flask to the mark, mix and filter.

11. Application examples

Determination of formic acid in fruit juices

Dilute fruit juices with a high formic acid content with water in a ratio of 1+1; use 0.100 ml for the assay. When analyzing strongly colored fruit juices 100 mg charcoal are added to 5 ml juice. After ca. 1 min of stirring, filter.

Adjust strongly acid juices to pH 7-8 with potassium hydroxide (1 M), if necessary (if high sample volumes are used).

Determination of formic acid in mixed pickles

Separate the liquid from the solid part, e.g. by filtration. For fat separation place the sample for 30 min in a refrigerator or for 20 min in an ice-bath, filter and dilute according to the dilution table.

Determination of formic acid in wine

Treat the sample as described under fruit juices. When analyzing red wine add 100 mg charcoal to 5 ml sample, stir for 1 min and filter. Use 0.200 ml for the assay.

Determination of formic acid in vinegar

Neutralize sample with potassium hydroxide (1 M) to pH 7-8. Dilute with water in a ratio of 1 + 1. Use 0.200 ml for the assay.

Determination of formic acid in fruit and vegetable products

Accurately weigh approx. 50 g homogenized vegetables into a 250 ml volumetric flask and add approx. 100 ml water. Stir the mixture for approx. 15 min (magnetic stirrer) in a closed flask. Fill up to the mark with water, mix, filter and centrifuge if necessary. Adjust strongly acidic sample solutions with a few drops of potassium hydroxide (1 M) to pH 7-8. Use 0.100 ml sample solution, diluted according to the dilution table, if necessary, for the assay.

Determination of formic acid in fish and meat products

Accurately weigh approx. 5 g homogenized sample into a homogenizer, add approx. 20 ml perchloric acid solution (1 M) and homogenize for 10 min. Transfer the mixture quantitatively into a beaker, adjust with potassium hydroxide (2 M) to pH 9-10 under stirring (magnetic stirrer). Transfer the mixture with approx. 20 ml water quantitatively into a 100 ml volumetric flask, fill up to the mark with redist. water. Take care that the fatty layer stands **above** the mark. Mix and place the mixture for separation of fat and for precipitation of potassium perchlorate for 20 min in a refrigerator. Filter, discard the first few ml and use the clear slightly turbid solution for the assay. For calculation of the amount of formic acid take the volume displacement factor of 0.98 into account.

Determination of formic acid in bakery goods (ref. 3.5)

Accurately weigh approx. 5 g of the homogenized sample into a 100 ml volumetric flask; add approx. 75 ml redist. water and extract at 20-25°C for 15 min. Fill up to the mark with water, mix and filter. Use the filtrate for the assay.

Determination of formic acid in honey (ref. 3.6-3.8)

Accurately weigh approx. 10 g honey into a 100 ml volumetric flask; fill up to the mark with redist. water and mix. Use the solution for the assay.

Determination of formic acid in jam

Accurately weigh approx. 20 g of the homogenized sample into a 100 ml beaker and add approx. 20 ml hot water (approx. 60°C). Subsequently add 2 ml Carrez-I-solution (15 g sodium hexacyano-ferrate(II) (ferrocyanide), $K_4[Fe(CN)_6] \times 3 H_2O/100$ ml) and 2 ml Carrez-II-solution (30 g zinc sulfate, $ZnSO_4 \times 7 H_2O/100$ ml). Adjust the pH to 7.5-8.5 with approx. 2 ml sodium hydroxide (1 M). Mix after each addition. Cool the solution to 20-25°C, transfer the liquid quantitatively to a 100 ml volumetric flask. Fill up to the mark, mix and filter. Use 0.500 ml of the filtrate for the determination.

Determination of formic acid in protein containing samples

To protein containing sample solutions add trichloroacetic acid (30 mM) in a ratio of 1 + 2, stir for 1 min, transfer into a volumetric flask and neutralize with potassium hydroxide (1 M), fill up to the mark with redist. water and filter. Use the clear solution, diluted, if necessary, for the assay.

12. Further applications

The method may also be used with the investigation of paper and cardboard (suggested sample preparation: extraction of formic acid with water at 60°C in a closed vessel; see Ref. 2.1). For the investigation of biological samples in research see Ref. 1.1 and 3.10

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Formic acid assay control solution

The assay control solution serves as a control for the enzymatic determination of formic acid in foodstuffs and other materials.

Reagents

Sodium formate, AR grade

Preparation of the assay control solution

Accurately weigh approx. 148 mg sodium formate to the nearest 0.1 mg into a 1000 ml volumetric flask, fill up to the mark with redist. water, and mix thoroughly (this corresponds to approx. 0.1 g formic acid/l).

Prepare assay control solution freshly before use. The assay control solution may be frozen in portions.

Application:

1. Addition of formic acid assay control solution to the assay mixture:

Instead of sample solution the assay control solution is used for the assay. (The measurement of the assay control solution is not necessary for calculating results.)

2. Restart of the reaction, quantitatively:

After completion of the reaction with sample solution and measuring of A_2 , add 0.050 ml assay control solution to the assay mixture. Read absorbance A_3 after the end of the reaction (approx. 20 min). Calculate the concentration from the difference of ($A_3 - A_2$) according to the general equation for calculating the concentration. The altered total volume must be taken into account. Because of the dilution of the assay mixture by the addition of the assay control solution, the result differs insignificantly from the result got according to pt. 1.

3. Internal standard:

The assay control solution can be used as an internal standard in order to check the determination for correct performance (gross errors) and to see whether the sample solution is free from interfering substances

Pipette into cuvettes	Blank	Sample	Standard	Sample + Standard
reaction mixture 2	1.000 ml	1.000 ml	1.000 ml	1.000 ml
sample solution	-	0.100 ml	-	0.050 ml
assay control sln.	-	-	0.100 ml	0.050 ml
redist. water	2.000 ml	1.900 ml	1.900 ml	1.900 ml

Mix, and read absorbances of the solutions (A_1) after approx. 5 min. Continue as described in the pipetting scheme under "Procedure". Follow the instructions given under "Instructions for performance of assay" and the footnotes.

$$\text{recovery} = \frac{2 \times \Delta A_{\text{sample + standard}} - \Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \times 100 [\%]$$

4. Recovery experiments with original samples:

For checking sample preparation and assay, recovery experiments may be carried out. For this, either the a. m. assay control solution is used or another assay control solution with a suitable concentration is prepared.

The original sample is measured with and without added sodium formate. The amount of added sodium formate

- is either the same as expected to be present in the original sample,
- or corresponds to that amount of formic acid which is allowed to be contained in the sample e.g. according to standards or other regulations.



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