BRITISH STANDARD

BS EN ISO
5377:1994

Starch hydrolysis products — Determination of reducing power and dextrose equivalent — Lane and Eynon constant titre method

The European Standard EN ISO 5377:1994 has the status of a British Standard

UDC 664.28:664.162.036:543.24

Cooperating organizations

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National foreword

This British Standard has been prepared under the direction of the BSI Standards Board and is the English language version of EN ISO 5377:1994 *Starch hydrolysis products — Determination of reducing power and dextrose equivalent — Lane and Eynon constant titre method*, published by the European Committee for Standardization (CEN). It is identical with ISO 5377:1981 *Starch hydrolysis products — Determination of reducing power and dextrose equivalent — Lane and Eynon constant titre method*, published by the International Organization for Standardization (ISO).

This British Standard has been produced to fulfil BSI's obligation to publish all approved European Standards but, because of the absence of interest in the UK in the subject concerned, there has been no UK participation in the preparation of EN ISO 5377.

Any queries relating to the EN should be directed to BSI.

A British Standard does not purport to include all the necessary provisions of a contract. Users of British Standards are responsible for their correct application.

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Summary of pages

This document comprises a front cover, an inside front cover, pages i and ii, the EN ISO title page, pages 2 to 8, an inside back cover and a back cover.

This standard has been updated (see copyright date) and may have had amendments incorporated. This will be indicated in the amendment table on the inside front cover.

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN ISO 5377

August 1994

UDC 664.28:664.162.036:543.24

Descriptors: Starches, tests, determination, mass losses, dry matter, titration

English version

Starch hydrolysis products — Determination of reducing power and dextrose equivalent — Lane and Eynon constant titre method

(ISO 5377:1981)

Produits d'hydrolyse de l'amidon ou de la fécule — Détermination du pouvoir réducteur et de l'équivalent en dextrose — Méthode Lane et Eynon à titre constant
(ISO 5377:1981)

Stärkehydrolyseprodukte — Bestimmung des Reduktionsvermögens und des Dextroseäquivalents — Verfahren mit konstantem Titer nach Lane und Eynon
(ISO 5377:1981)

This European Standard was approved by CEN on 1994-08-22. CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration.

Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

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CEN

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

Central Secretariat: rue de Stassart 36, B-1050 Brussels

Foreword

The text of the International Standard ISO 5377:1981, prepared by ISO/TC 93, Starch, was submitted to the formal vote and was approved by CEN as EN ISO 5377:1994 on 1994-08-22 without any modifications.

This European Standard shall be given the status of a national standard, either by publication or by endorsement, at the latest by February 1995, and conflicting national standards shall be withdrawn at the latest by February 1995.

According to the CEN/CENELEC Internal Regulations, the following countries are bound to implement this European Standard: Austria, Belgium, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland, United Kingdom.

1 Scope and field of application

This International Standard specifies a Lane and Eynon constant titre method for the determination of the reducing power and dextrose equivalent of all starch hydrolysis products.

2 References

ISO 385-1, *Laboratory glassware — Burettes — Part 1: General requirements*[1].

ISO 385-2, *Laboratory glassware — Part 2: Burettes for which no waiting time is specified*[1].

ISO 648, *Laboratory glassware — One-mark pipettes.*

ISO 1042, *Laboratory glassware — One-mark volumetric flasks.*

ISO 1741, *Dextrose — Determination of loss in mass on drying — Vacuum oven method.*

ISO 1742, *Glucose syrup — Determination of dry matter content — Vacuum oven method.*

ISO 1743, *Glucose syrup and dextrose — Determination of dry matter content — Refractive index method.*

ISO 1773, *Laboratory glassware — Boiling flasks (narrow-necked).*

ISO 5809, *Starch, including derivatives and by-products — Determination of sulphated ash*[2].

3 Definitions

3.1
reducing power

the content of reducing sugars, expressed as the number of grams of anhydrous D-glucose per 100 g of the sample, when determined by the method specified in this International Standard

3.2
dextrose equivalent

the content of reducing sugars, expressed as the number of grams of anhydrous D-glucose per 100 g of the dry matter in the sample, when determined by the method specified in this International Standard

4 Principle

Titration of a prescribed volume of mixed Fehling's solution with a solution of a test portion under specified conditions, using methylene blue as internal indicator.

5 Reagents

During the analysis, use only reagents of recognized analytical grade and only distilled water or water of equivalent purity.

5.1 *Fehling's stock solutions*

Prepare the following solutions, using apparatus in accordance with clause **6**.

5.1.1 *Stock solution A*

Copper(II) sulphate pentahydrate ($CuSO_4.5H_2O$)	69,3 g
Water to	1 000,0 ml

5.1.2 *Stock solution B*

Potassium sodium tartrate tetrahydrate ($KNaC_4H_4O_6.4H_2O$)	346,0 g
Sodium hydroxide (NaOH)	100,0 g
Water to	1 000,0 ml

Before use, decant the clear solution from any sediment that may form.

5.1.3 *Mixed Fehling's solution*

Transfer into a dry stock glass bottle, in the following order, 100 ml of solution A (**5.1.1**) and 100 ml of solution B (**5.1.2**). Mix well.

NOTE Do not keep Fehling's solution. Prepare this mixed solution just before use and standardize it as specified in **7.1**.

5.2 *Anhydrous D-glucose,* complying with the following requirements:

a) a solution containing 400 g/l shall be free from haze and sediment and shall show no more colour than the water used in its preparation when examined downwards in 50 ml Nessler tubes (**6.5**) filled to the marks;

b) the sulphated ash content shall be not greater than 0,01 % (*m*/*m*) when determined by the method specified in ISO 5809 amended as follows:

1) the mass of the test portion shall be increased to 20 g,

2) only a platinum dish shall be used for incineration,

3) the platinum dish shall be weighed to the nearest 0,1 mg before and after the incineration;

c) the maltose and/or *iso*maltose content shall not exceed 0,1 % (*m*/*m*) and no sugar of greater relative molecular mass shall be detected.

[1] At present at the stage of draft. (Revision, in part, of ISO/R 385.)

[2] At present at the stage of draft.

5.3 *D-glucose,* standard reference solution.

5.3.1 Determine the dry matter content of the anhydrous D-glucose by the method specified in ISO 1741.

5.3.2 Weigh, to the nearest 0,1 mg, a mass of the anhydrous D-glucose (**5.2**) containing 0,600 g of solids, dissolve it in water, transfer the solution quantitively into a 100 ml one-mark volumetric flask (**6.4**), dilute to the mark with water and mix.

Prepare this solution freshly on each day of use.

5.4 *Methylene blue* ($C_{16}H_{18}ClN_3S.2H_2O$), indicator, 1 g/l aqueous solution.

6 Apparatus

Ordinary laboratory apparatus and in particular

6.1 *Narrow-necked boiling flask*, of capacity 250 ml, complying with the requirements of ISO 1773.

6.2 *Burette*, of capacity 25 ml, graduated in subdivisions of 0,05 ml, complying with the requirements of ISO 385-2, class A.

6.3 *One-mark pipettes*, of capacity 1 ml and 25 ml, complying with the requirements of ISO 648, class A.

6.4 *One-mark volumetric flasks*, of capacity 100 ml, 500 ml and 1 000 ml, complying with the requirements of ISO 1042, class A.

6.5 *Nessler tubes*, of capacity 50 ml.

6.6 *Heating device*, suitable for maintaining boiling as required in **7.1.4** whilst enabling the colour change at the end-point of the titration to be observed without the flask having to be removed from the heating device.

6.7 *Stop-watch*

7 Procedure

NOTE 1 A boiling aid (for example glass beads) may be added, if desired, to ensure freedom from super-heating.
NOTE 2 Shield the burette from the source of heat (**6.6**) at all times.

7.1 Standardization of the mixed Fehling's solution (5.1.3)

7.1.1 Using a pipette (**6.3**), place 25,0 ml of the Fehling's solution (**5.1.3**) in the clean, dry boiling flask (**6.1**).

7.1.2 Fill the burette (**6.2**) to the zero mark with the D-glucose standard reference solution (**5.3**).

7.1.3 Run into the boiling flask from the burette, 18,0 ml of the D-glucose solution (**5.3**). Swirl the flask to mix the contents.

7.1.4 Place the boiling flask on the heating device (**6.6**), previously adjusted so that the boiling commences in 120 ± 15 s as timed by the stop-watch (**6.7**).

Do not adjust the heating device subsequently. This ensures that once boiling has commenced the evolution of steam is brisk and continuous throughout the whole of the titration process, thus preventing to the maximum possible extent the entrance of air to the titration flask with consequent re-oxidation of its contents.

7.1.5 Bring the contents of the flask to the boil and continue boiling for 120 s (timed by the stop-watch). Add 1 ml of the methylene blue solution (**5.4**) towards the end of this period. After expiry of the 120 s period, commence adding the D-glucose solution to the flask from the burette in 0,5 ml increments until the colour of the methylene blue is discharged, boiling being continued during the whole titration.

Note the total volume of the D-glucose solution (V ml), added up to and including the penultimate 0,5 ml increment.

NOTE The disappearance of the colour of the methylene blue is best seen by looking at the upper layers and the meniscus of the contents of the titration flask, as these will be relatively free from the precipitated, red copper(I) oxide. The colour disappearance is more easily seen when indirect lighting is used. A white screen behind the titration flask is helpful.

7.1.6 Repeat **7.1.1** and **7.1.2**.

7.1.7 From the burette run into the boiling flask a volume of the D-glucose solution equal to ($V - 0,3$) ml.

7.1.8 Repeat **7.1.4**.

7.1.9 Bring the contents of the flask to the boil and continue boiling for 120 s (timed by the stop-watch). Add 1 ml of the methylene blue solution towards the end of this period. After expiry of the 120 s period, commence adding the D-glucose solution to the flask from the burette, initially in 0,2 ml increments and finally drop by drop, until the colour of the methylene blue (**5.4**) is just discharged, boiling being continued during the whole titration.

Towards the end of this action, add the successive increments of the D-glucose solution at intervals of 10 to 15 s. Complete these additions within 60 s to give a total time of boiling not longer than 180 s.

A third titration with a slightly larger, appropriately adjusted, initial addition of the D-glucose solution may be necessary to achieve this.

7.1.10 Note the volume of the D-glucose solution used up to the end-point of the final titration.

7.1.11 It is essential that the titre is between 19,0 and 21,0 ml of the D-glucose solution. If it is outside these limits, adjust the concentration of the Fehling's solution A (**5.1.1**) appropriately and repeat the standardization process.

7.1.12 Repeat **7.1.6** to **7.1.10** and calculate the mean of the two titres (V_1 ml).

7.1.13 For the day-to-day standardization of the mixed Fehling's solution, as V_1 is known with accuracy, a single titration only is necessary, using an initial addition of (V_1 – 0,5) ml of the D-glucose solution.

NOTE As there is a personal factor involved, it is essential that each operator carries out his own standardization titration and uses his own value of V_1 in the calculation (**8.1.1**).

7.2 Determination

7.2.1 *Preparation of test sample*

If the sample is in powder or crystalline form, remove it from its container, break down any lumps, mix in an appropriate manner and place in a suitable airtight container.

If the sample is in massive, solid form, e.g. starch sugar (solid glucose), melt it in a closed container immersed in a hot water bath at 60 to 70 °C, allow to cool to ambient temperature and shake a number of times without removing the closure, in order to mix any condensed moisture on the interior into the sample.

If the sample is in liquid form, mix it by stirring it in its container, after removing any skin that may have formed on its surface.

7.2.2 If the reducing sugar content of the sample is unknown, obtain an approximation to its value by prior trial titrations, in general as specified in **7.1.1** to **7.1.5**, but with the following modifications:

a) Add 10,0 ml of the test solution in place of the D-glucose solution added in **7.1.3**.

b) After **7.1.4**

1) Immediately boiling starts, add 1 ml of the methylene blue solution and commence adding the test solution to the flask from the burette in 1,0 ml increments at intervals of approximately 10 s until the blue colour of the methylene blue is discharged. If the blue colour is discharged before the addition of any 1,0 ml increments of test solution, decrease the concentration of the test solution and repeat the titration.

2) Note the total volume of test solution (V' ml), added up to and including the penultimate increment.

V' shall not be greater than 50 ml. If it is, increase the concentration of the test solution and repeat the titration.

3) The approximate reducing power (see **3.1**) (ARP) of the test sample is given by the formula

$$\text{ARP} = \frac{F \times 100 \times 500}{V' \times m_0}$$

$$= \frac{50\,000 \times F}{V' \times m_0}$$

$$= \frac{300 \times V_1}{V' \times m_0}$$

where

$F = (0{,}6 \times V_1)/100$

$= 0{,}006 \times V_1$

m_0 is the mass, in grams, of the test portion in 500 ml of test solution.

The mass, in grams, of the test portion to be taken is

$$\frac{100 \times 3}{\text{ARP}} = \frac{300}{\text{ARP}}$$

7.2.3 *Test portion*

Weigh, to the nearest 1 mg, a mass of the test sample (m g) containing between 2,85 and 3,15 g of reducing sugars (expressed as anhydrous D-glucose).

7.2.4 *Test solution*

Dissolve the test portion (**7.2.3**) in water, transfer the solution quantitively to a 500 ml one-mark volumetric flask (**6.4**), dilute to the mark with water and mix.

7.2.5 *Titration*

7.2.5.1 Repeat **7.1.1** to **7.1.9** using the test solution (**7.2.4**) in place of the D-glucose solution (**5.3**).

7.2.5.2 Note the volume (V_2) of the test solution (**7.2.4**) used up to the end-point of the final titration.

7.2.5.3 It is essential that V_2 is between 19,0 and 21,0 ml of the test solution (**7.2.4**). If V_2 lies outside these limits, adjust the concentration of the test solution appropriately and repeat **7.2.5.1** and **7.2.5.2**.

7.2.5.4 Carry out two determinations on the same test sample.

7.3 Dry matter content

Determine the dry matter content [DMC % (*m*/*m*)] of the test sample as follows:

a) For dried glucose syrup, by the method specified in ISO 1742.

b) For dextrose (anhydrous and monohydrate), by the method specified in ISO 1741.

c) For glucose syrup, by the method specified in ISO 1743.

8 Expression of results

8.1 Method of calculation and formulae

8.1.1 ***Reducing power (Lane and Eynon)*** (see **3.1**)

$$RP = \frac{0{,}600 \times V_1}{100} \times \frac{500}{V_2} \times \frac{100}{m}$$

$$= \frac{300 \times V_1}{V_2 \times m}$$

8.1.2 ***Dextrose equivalent (Lane and Eynon)*** (see **3.2**)

$$DE = \frac{RP \times 100}{DMC}$$

where

V_1 is the volume, in millilitres, of the D-glucose solution (**5.3**) used in the standardization of the mixed Fehling's solution (**7.1**);

V_2 is the volume, in millilitres, of the test solution (**7.2.4**) used in the determination (**7.2.5**);

m is the mass, in grams, of the test portion (**7.2.3**) used to make 500 ml of the test solution (**7.2.4**);

DMC is the dry matter content of the test sample, expressed as a percentage by mass (see **7.3**).

8.1.3 Report the result as the arithmetic mean of the two determinations, provided that the requirement concerning repeatability (see **8.2**) is satisfied.

8.2 Repeatability

The results of two determinations carried out in rapid succession by the same analyst shall not differ by more than 0,75 % of their arithmetic mean.

8.3 Reproducibility

The results reported by two different laboratories on the same test sample shall not differ by more than 1,5 % of their arithmetic mean.

9 Test report

The test report shall include all details required for complete identification of the sample, the date of test, the number of this International Standard, the method used, the results obtained and any operating conditions not described in this International Standard (or regarded as optional) as well as any circumstances that might have influenced the results.

Annex Method for testing standard reference anhydrous D-glucose for compliance with the requirements in 5.2 c)

(This annex is given for information purposes only.)

A.1 Principle

Separation of the maltose and sugars of greater relative molecular mass from a known mass of the sample of anhydrous D-glucose by paper chromatography. Detection of the separated sugars as coloured spots by dipping the developed chromatogram in a solution of a colour-forming reagent and heating.

Verification that the maltose (as anhydrous) content does not exceed 0,10 % (*m*/*m*) by visual comparison of the intensities of the maltose spot from the dextrose and a standard maltose spot. Visual examination for the presence of spots of sugars other than D-glucose and maltose.

NOTE Maltose and *iso*maltose are not resolved by the specified method; references to "maltose" should therefore be understood to mean "maltose and/or *iso*maltose".

A.2 Reagents

During the analysis, use only reagents of recognized analytical grade and only distilled water or water of equivalent purity.

A.2.1 *Paper for chromatography:* Pure cotton cellulose, 185 g/m^2, with an α-cellulose content greater than 97 % (*m*/*m*) and ash of less than 0,06 % (*m*/*m*), both calculated on the dry basis, and of minimum dimensions 140 mm wide × 420 mm long in the direction of solvent travel.

NOTE Whatman No. 3 Chromatography Paper is suitable.

A.2.2 *Development solvent*

n-Propanol	7 volumes
Ethyl acetate	1 volume
Water	2 volumes

Prepare fresh on each day of use.

A.2.3 *Colour reagent*

Diphenylamine	1 g
Aniline	1 ml
Acetone	100 ml
Orthophosphoric acid (H_3PO_4) 88 % (*m*/*m*)	6 ml

Dissolve the diphenylamine and the aniline in the acetone, add the orthophosphoric acid and mix.

Prepare freshly on each day of use.

A.2.4 *Maltose hydrate*

10 µl of a solution containing 20 g/l of anhydrous maltose shall show no spot other than that of maltose when treated as in **A.4.4**, **A.4.5** and **A.4.6**.

Sugars other than maltose are then present in quantities not exceeding 1,0 % (*m*/*m*) (on anhydrous maltose) (see **A.5.3**).

A.2.5 *Maltose,* standard solution corresponding to 5 g of anhydrous maltose per litre.

Weigh, to the nearest 1 mg, 0,526 g of the maltose hydrate (**A.2.4**), equivalent to 0,500 g of anhydrous maltose, dissolve it in water, transfer the solution quantitatively to a 100 ml one-mark volumetric flask (**A.3.1**), dilute to the mark with water and mix.

1 ml of this standard solution contains 0,005 g of anhydrous maltose.

Prepare this solution freshly on each day of use.

A.2.6 *Maltose,* standard solution corresponding to 0,5 g of anhydrous maltose per litre.

Using a pipette (**A.3.2**), introduce 10,0 ml of the standard maltose solution (**A.2.5**) into a 100 ml one-mark volumetric flask (**A.3.1**), dilute to the mark with water and mix.

1 ml of this standard solution contains 0,5 mg of anhydrous maltose.

Prepare this solution freshly on each day of use.

A.3 Apparatus

A.3.1 *One-mark volumetric flasks*, of capacity 100 ml, complying with the requirements of ISO 1042, class B.

A.3.2 *One-mark pipette*, of capacity 10 ml, complying with the requirements of ISO 648, class B.

A.3.3 *Micropipettes*, to deliver 10 µl.

A.3.4 *Domestic hair dryer* or other suitable sources of a current of warm air at a temperature not greater than 40 °C.

A.3.5 *Chromatography tank*, for downward development.

A.3.6 *Trough*, suitable for dipping the chromatography paper (**A.4.6.1**) in the colour reagent (**A.2.3**).

A.3.7 *Forced circulation hot-air oven*, of a size suitable for heating the chromatography paper (**A.4.6.3**), and capable of being controlled at 80 ± 1 °C.

A.4 Procedure

A.4.1 *Test sample*

Mix the sample thoroughly.

A.4.2 *Test solution*

Prepare freshly on each day of use a solution containing 250 g of the test sample (**A.4.1**) per litre.

A.4.3 *Preparation of the chromatography paper*

A.4.3.1 Draw in lead pencil on the chromatography paper (**A.2.1**) an origin line, parallel to the 140 mm edge and 80 mm from it.

NOTE The distance of 80 mm may have to be varied to suit the particular chromatography tank (**A.3.5**) being used.

A.4.3.2 Mark off on this line, in lead pencil, five origin spots, A, B, C, D and E, 20 mm apart, starting 30 mm from a 420 mm edge.

A.4.4 *Application of the test and standard maltose solutions*

A.4.4.1 At each of the origin spots B and D (**A.4.3.2**) apply by micropipette (**A.3.3**) two 10 µl increments (containing 5 000 µg of anhydrous D-glucose) of the test solution (**A.4.2**), drying the spots in a current of warm air (see **A.3.4**) immediately after each application.

A.4.4.2 At each of the origin spots A, C and E apply by micropipette (**A.3.3**) 10 µl (containing 5 µg of anhydrous maltose) of the standard maltose solution (**A.2.6**), drying each spot in a current of warm air immediately after the application of the solution.

A.4.5 *Development of the chromatogram*

A.4.5.1 Place the spotted paper (**A.4.4**) in the equilibriated chromatography tank (**A.3.5**) containing the development solvent (**A.2.2**).

NOTE The chromatography tank should be in a dark, draught-free space, preferably maintained at a constant temperature.

A.4.5.2 Allow the chromatogram to develop for 8 h. This time is suitable at a temperature of 20 °C but may have to be decreased if the temperature is appreciably higher. It is essential that the centre of the D-glucose spot (see **A.4.6**) has moved at least 50 mm from its origin but has not been washed off the chromatogram.

A.4.5.3 Remove the paper from the chromatography tank and dry it in a current of warm air.

NOTE The paper may also be dried in a suitable drying oven maintained at a temperature not greater than 40 °C.

A.4.6 *Detection of the sugar spots*

A.4.6.1 Pass the developed, dry chromatogram (**A.4.5.3**) at a uniform rate through the trough (**A.3.6**) containing the colour reagent (**A.2.3**).

A.4.6.2 Drain the chromatogram and dry it in a current of warm air (see **A.3.4**) or in a suitable drying oven (see the note in **A.4.5.3**).

A.4.6.3 Place the dried chromatogram (**A.4.6.2**) in the forced-circulation hot-air oven (**A.3.7**).

A.4.6.4 Examine the chromatogram after 5 min and thereafter at frequent intervals. Remove it from the oven when the coloured spots are clearly defined but the chromatogram as a whole is uncoloured.

A.4.7 *Examination of the chromatogram*

A.4.7.1 Assess visually the relative intensities of colour of the maltose spots from origin spots B and D (**A.4.4.1**, test solution) and the maltose spots from origin spots A, C and E (**A.4.4.2**, standard maltose solution).

A.4.7.2 Examine the chromatogram visually for evidence of sugars other than D-glucose and maltose from origin spots B and D.

A.5 Assessment of results

A.5.1 If the maltose spots from origin spots B and D (**A.4.4.1**) have a colour that is no more intense than that of the spots from origin spots A, C, and E (**A.4.4.2**) the maltose content (as anhydrous maltose) of the sample of anhydrous D-glucose is not greater than 0,1 % (*m*/*m*).

A.5.2 If the only coloured spots from origin spots B and D (**A.4.4.1**) are those of D-glucose and maltose and the remainder of the chromatogram is free from colour, then the sample of anhydrous D-glucose satisfies the requirement for the absence of sugars of greater relative molecular mass than maltose.

A.6 Sensitivity of the method

The smallest mass of anhydrous maltose which will give a coloured spot that is just visible is 2 µg.

National annex NA (informative)
Cross-references

Publication referred to	Corresponding British Standard
ISO 1042:1983	BS 1792:1982 *Specification for one-mark volumetric flasks*
ISO 1741:1980	BS EN 21741:1994 *Dextrose — Determination of loss in mass on drying — Vacuum oven method*
ISO 1773:1976	BS 2734:1984 *Specification for boiling flasks (narrow-necked), conical flat bottom and round bottom*
ISO 5809:1982	BS EN 25809:1994 *Starches and derived products — Determination of sulfated ash*

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