**DRAFT KENYA STANDARD DKS 2041: 2024**

ICS 71.080.60

**Denatured Ethanol for industrial use - Specification**

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**DRAFT KENYA STANDARD**

**DKS 2041:2024**

ICS 71.080.60

**Ethanol for industrial use - Specification**

**KENYA BUREAU OF STANDARDS (KEBS)**

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**Foreword**

This Kenya Standard has been prepared by the Technical Committee on Industrial Solvents and Chemicals under the guidance of the Chemical Industry Standards Committee, and it is in accordance with the procedures of the Kenya Bureau of Standards.

Denatured Ethanol for industrial use is used as a raw material for many Industrial process e.g. in the manufacture of industrial solvents, and for further processing into other finished products.

The standard covers important parameters such as, strength, colour, and residue on evaporation, miscibility with water, alkalinity or acidity among others.

During the preparation of this standard, reference was made to the following documents: BS 507 Specification for ethanol for industrial use.

GT/T 394.1-94 Ethanol for industrial use.

Acknowledgment is hereby made for the assistance derived from this sources.

**KENYA STANDARD DKS 2041: 2024**

**Denatured Ethanol for industrial use - Specification**

**1 Scope**

This Kenya Standard specifies the requirements and test methods for denatured ethanol suitable for industrial use. The standard shall apply to hydrous ethanol which has been produced by the fermentation of cereals, potatoes or molasses. It does not apply to material for medical and beverage use.

**2 Normative references**

The following documents are indispensable for the application of this standard. For dated references, only the edition cited applies. For undated reference, the latest edition of the referenced document (including any amendments) applies:

KS ISO 1388-1:1981 *Ethanol for industrial use - Methods of test - Part 1: General*

KS ISO 1388-2:1981 *Ethanol for industrial use - Methods of test --Part 2: Detection of alkalinity or determination of acidity to phenolphthalein*

KS ISO 1388-3:1981 *Ethanol for industrial use - Methods of test - Part 3: Estimation of content of carbonyl compounds present in small amounts - Photometric method*

KS ISO 1388-4:1981 *Ethanol for industrial use - Methods of test - Part 4: Estimation of content of carbonyl compounds present in moderate amounts - Titrimetric method*

KS ISO 1388-12:1981 *Ethanol for industrial use - Methods of test - Part 12: Determination of permanganate time*

ISO 1388-5:1981 *Ethanol for industrial use - Methods of test - Part 5: Determination of aldehydes content*

*- Visual calorimetric method*

KS ISO 1388-8:1981 *Ethanol for industrial use - Methods of test - Part 8: Determination of methanol content [methanol contents between 0.10 and 1.50 % (V/V) - Visual colorimetric method*

TES/04/TM/39 *Determination of congeners in alcoholic beverages by direct injectioni on GC-FID*

TES/04/TM/36 *(Determination of Ethyl Alcohol (Ethanol) content by distillation method and volatile acidity in strong alcoholic beverages)*

**3 Terms and definitions**

For the purposes of this document, the following terms and definitions apply.

**3.1 Denaturant**

A completely miscible chemical substance added to alcohol to render it unpalatable and unfit for human consumption

**3.2 Denatured Industrial Ethanol**

Industrial Ethanol shall be denatured by the addition of Denatonium Benzoate

**3.3 Grades**

This standard covers four grades of denatured industrial ethanol, namely:

Grade 1:

Grade 2:

Grade 3:

Grade 4:

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**4 Requirements**

**4.1 Description**

The material shall be clear for grades 1, 2, and 3 or primrose for grade 4. It shall be free from matter in suspension, as assessed by visual inspection, and shall consist, apart from water, essentially of ethanol, C2H5OH.

**4.2 Chemical requirements**

Denatured Ethanol for industrial use shall comply with the requirements given in Table 1.

**Table 1 - Chemical requirement for denatured ethanol for industrial use**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **SL No.** | **Parameter** | **Requirement** | | | | **Test method** |
| Grade 1 | Grade 2 | Grade 3 | Grade 4 |
| i) | Odour | No foreign | No foreign | No foreign | **-** |  |
| odour | odour | odour |  |
| ii) | Ethanol content, %  v/v, min. | 96.0 | 95.5 | 95.0 | 95.0 | ANNEX B |
| iii) | Permanganate | 30 | 15 | 5 | **-** | KS ISO 1388- |
| time, minutes, min. | 12:1981 |
| iv) | Aldehydes, as | 5 | 30 | **-** | **-** | KS ISO 1388- |
| acetaldehyde, | 3,4,5:1981 |
| mg/litre, max. |  |
| v) | Fusel oil, as  isobutanol plus isoamyl alcohol, mg/litre, max. | 10 | 80 | 400 | **-** | ANNEX A |
| vi) | Methanol, mg/litre, | 800 | 1 200 | 2 000 | **-** | KS ISO 1388- |
| max. | 8:1981 |
| vii) | Acidity, as acetic  acid, mg/litre, max. | 10 | 20 | 20 | **-** | KS ISO 1388-  2:1981 |
| viii) | Esters, as ethyl acetate, mg/litre,  max. | 30 | 40 | **-** | **-** | ANNEX A |
| ix) | Non-volatile | 20 | 25 | 25 | **-** | KS ISO 1388- |
| residue, mg/litre, | 1:1981 |
| x) | Denatonium Benzoate |  |  |  |  | ANNEX C |

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**5 Sampling and size of sample**

A representative sample of the material measuring not less than 2 500 mL shall be taken from the bulk for the purpose of examination in accordance with this standard. The sample shall be placed in a clean, dry and air-tight, ground glass-stoppered bottle of such capacity that it is almost filled by the sample. When it is necessary to seal the container, care shall be taken to avoid the risk of contaminating the contents in any way.

**6 Miscibility within water**

The material shall not show opalescence when diluted 1 + 19 by volume of distilled water and tested in accordance with KS ISO 1388-6:1981.

**7 Packaging and marking**

**7.1 Packaging**

The denatured industrial ethanol shall be packed in suitable containers in the following measurers: bulk, 20 L, 4 L, 2.5 L, 1 L, ½ L and ¼ L.

**7.2 Marking**

Each container shall be marked legibly and indelibly with the following:

|  |
| --- |
| 1. The words, 'Denatured ethanol suitable for industrial use'; |
| 1. Name and address of the manufacturer and/or registered trademark. 2. Volume of the material, in L; |
| 1. Instructions for use, disposal and safety precautions. 2. Expiry date. 3. Date of manufacture; |
| 1. Country of origin. 2. Name and %v/v of denaturant |

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**ANNEX A**

(Normative)

**DETERMINATION OF CONGENERS IN ALCOHOLIC BEVERAGES BY DIRECT INJECTION ON GC – FID**

**A.1 Principle**

Alcoholic beverages are injected directly (neat) into the gas chromatograph (GC) for separation and the analytes are detected using the flame ionization detector (FID).

**A.2 Reagents**

1. All reagents shall be of HPLC grade or better. The purity must be declared.
2. All gases shall be instruments grade and a gas filter shall be fitted.

# **A.3** **Apparatus**

1. Analytical balance with an accuracy of ±0.0001g
2. Gas-tight syringes 1mL, 2.5mL, 5.0mL, 25.0mL.
3. Clear glass vials with PFTE caps, 20 Ml
4. Measuring cylinder, 100 mL
5. Gas chromatograph equipped with flame ionization detector (GC-FID)
6. Fused Silica Capillary Column, 60m x 0.32mm x 0.5µm film thickness
7. 10µL GC injection Syringe
8. Micro syringe 10mL
9. Instrument grade H2, N2, and Compressed Air gases

# **A.4 Preparation of Calibration Standards**

All standards shall be prepared as per the procedure for gravimetric preparation of calibration standards

# **A.5** **GAS CHROMATOGRAPH TEST CONDITIONS**

1. Detector type: FID
2. Injection volume: 1µL
3. Injection type: split
4. split ratio: 20
5. Injector temperature: 250°C
6. Detector temperature: 200°C
7. Stabilization time: 0.01s

**A5.1**  **Oven temperature program:**

**15°C/min**  **40°C/min**

**35°C (3 min) 80°C 200°C (1 min)**

**(3 min)**  **(3 min)**

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**A.6 Elution order for Congeners**

1. Acetaldehyde
2. Ethyl acetate
3. Methanol
4. tert-butyl alcohol
5. iso-propyl alcohol
6. Ethanol
7. n-propanol
8. Amyl alcohol
9. Furfural

# **A.7 PERFORMANCE**

A.7.1 Establish the retention time window for each analyte of interest

A.7.2 All samples shall be injected (neat) directly into the gas chromatograph (GC) for screening to establish presence in the sample.

A7.3 If an analyte of interest is present in the sample, initiate the quantitative analysis of congeners procedure.

A.7.4 Ensure that you rinse the syringe thoroughly before and after injection of samples and standards to avoid cross contamination.

A.7.5 Run one standard before screening samples to ensure that retention times haven’t changed.

# **A.8** **PROCEDURE**

# **A.8.1** **Screening Method for Congeners**

# A.8.1. Inject 1µL of a reference standard mixture to ascertain that the congeners still elute within the established retention windows.

A.8.2 After the retention windows are confirmed, run the sample screening method to establish the presence of congeners in the samples.

A.8.3 Inject 1µL of each sample at least three times. When an analyte of interest peak is detected, it must appear in at least two out of three results to initiate the quantitative and confirmatory test.

A.8.4 If no peak corresponding to the congeners is present in all the three results of a sample, then you report “not detected”. However, if one peak corresponding to the congeners is detected, then you make three additional injections of the sample to confirm presence.

A.8.5 If two or more peaks corresponding to the retention time of the sample are detected, then initiate the quantitative test procedure for confirmation and quantification of the specific congeners.

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## **A.8.2 Quantitative Method for Congeners**

A.8.1 Weigh 0.5 mL of standards into autosampler vials and weigh 0.5 mL of internal standard into the same vial. Mix thoroughly by vortex.

A.8.2 Inject the calibration standards prepared using the procedure for gravimetric preparation of calibration standards for the specific congeners detected.

A.8.3 Record the corresponding peak areas for each concentration level of the standard and prepare a calibration curve.

A.8.4 Check the linearity of the calibration curve to ensure it is as close to 1 as possible. If the linearity is acceptable then you can use the equation of a straight line for quantification of the specific congener.

A.8.5 Calculate the relative response factor (RRF) for the calibration curve.

A.8.6 Prepare the samples in the same way as the standard in 10.2.1 above.

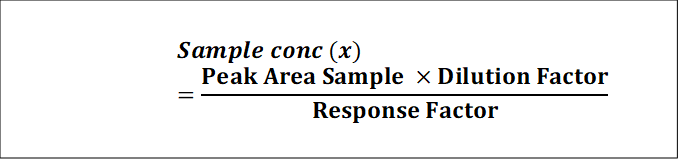
A.8.7 Inject the samples and record their peak area ratios.

A.8.8 Use Microsoft Excel for the quantification of specific congeners considering the dilution factors, if any.

# **A.9** **CALCULATION AND EXPRESSION OF RESULTS**

# **A.9.1** **External Standard Calibration Method**

Concentration from External Standard Calibration Method is calculated using the following formula:



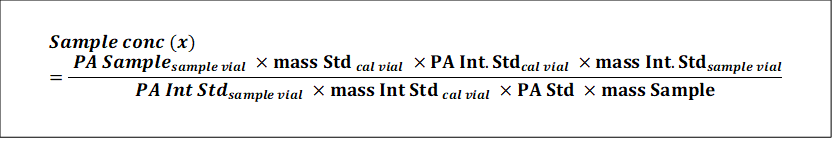
**Note:**

1. Sample dilution factor is determined gravimetrically and is equal to 1 when the sample is not diluted.
2. Response factor is the slope (gradient) of the calibration curve
3. This calculation is done using Microsoft Excel.

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# **A.9.2** **Internal Standard Calibration Method**

Concentration from Internal Standard Calibration Method is calculated using the following formula:



**A.9.3**  **Expression of results**

Levels ³ 10 mg/Kg but < 100 mg/Kg to the nearest three significant figures

Levels ³ 100 mg/Kg to the nearest whole number

Levels > 0.1 mg/Kg < 10mg/Kg to the nearest two significant figures.

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**ANNEX B**

(Normative)

**DETERMINATION OF ETHYL ALCOHOL (ETHANOL) CONTENT BY DISTILLATION METHOD**

**B.1 PRINCIPLE**

The apparent density in air of the spirit is determined by determining the mass of spirit required to fill the volume of the pycnometer, all measurements being done at 20°C.

The ethanol content is determined from the real density in air at 20°C by reference to the specific gravity/ethyl alcohol content tables in the AOAC.

**B.2 Reagents**

B.2.1 Standard Sodium hydroxide – 0.01M

B.2.2 1% Phenolphthalein indicator. (Prepared by dissolving 1g of phenolphthalein powder in 100 mL of 95% rectified spirit).

**B.3 Apparatus**

Ordinary laboratory apparatus and:

**B.3.1 Distillation assembly**.

**B.3.2 Receiver**: 250 mL capacity measuring cylinder.

**B.3.3 Pycnometer**

**B.3.4 Thermometer** – with a range of 0° to 30°C and with subdivisions at every 0.5°C shall be used.

**B.3.5 Measuring cylinder** - 100 mL.

**B.3.6 Densitometer**

# **B.4** **PROCEDURE**

**B.4.1** **Sample distillation**

B.4.1.1 Measure 100 ml of test portion using the measuring cylinder (6.2.5) and pour it into a 500 ml distillation flask.

B.4.1.2 Rinse the measuring cylinder with 2 portions of 100 ml distilled water and add to the distillation flask.

B.4.1.3 Add a few crystals of anti-bumping granules and distil gently collecting the distillate until all the alcohol has distilled over\*. (\*All alcohol will be assumed to have distilled over after 160 mL of distillate had been collected)

B.4.1.4 Transfer to a 200 ml volumetric flask.

B.4.1.5 Rinse the collector flask three times with distilled water transferring the washings to the volumetric flask.

B.4.1.6 Cool the distillate in the volumetric flask to about 20°C ± 0.5°C and top up to the mark with distilled water1.

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**B.4.2 Determination of specific gravity using Pycnometer method**

B.4.2.1 Weigh the empty pycnometer on an analytical balance to the nearest 0.0001 g.

B.4.2.2 Fill the pycnometer with distilled water at 20°C. Dip the thermometer into the water and ensure that the temperature reads exactly 20±0.5°C. Wipe the overflow with a paper towel and cover the overflow arm with the cap.

B.4.2.3 Weigh the pycnometer with the distilled water thus filled at 20±0.5°C on an analytical to the nearest 0.0001g

B.4.2.4 Repeat the procedure in steps 8.2.2 and 8.2.3. but this time with the alcohol distillate instead of distilled water.

B.4.2.5 Calculate the net mass in grams of the distilled water and the alcohol distillate at 20±0.5°C. in the pycnometer by subtracting the mass of the empty pycnometer.

B.4.2.6 Divide the mass of the alcohol distillate by the mass of distilled water which is given by the formula below:

**Specific gravity at 20°C/20°C = B – C**

**A - C**

*Where:*

*A is the mass of the pycnometer + distilled water.*

*B is the mass of the pycnometer + alcohol distillate*

*C is the mass of the pyknometer*[[1]](https://euc-word-edit.officeapps.live.com/we/wordeditorframe.aspx?ui=en-US&rs=en-US&wopisrc=https%3A%2F%2Fkebs770-my.sharepoint.com%2Fpersonal%2Fkitumf_kebs_org%2F_vti_bin%2Fwopi.ashx%2Ffiles%2Fb5040ef520bb421780bb56024ce3dff6&wdenableroaming=1&mscc=1&wdodb=1&hid=16373BA1-906F-9000-6004-12412042CAFA.0&uih=sharepointcom&wdlcid=en-US&jsapi=1&jsapiver=v2&corrid=f3c3211e-f448-d52b-9108-debab75b64f3&usid=f3c3211e-f448-d52b-9108-debab75b64f3&newsession=1&sftc=1&uihit=docaspx&muv=1&cac=1&sams=1&mtf=1&sfp=1&sdp=1&hch=1&hwfh=1&dchat=1&sc=%7B%22pmo%22%3A%22https%3A%2F%2Fkebs770-my.sharepoint.com%22%2C%22pmshare%22%3Atrue%7D&ctp=LeastProtected&rct=Normal&wdorigin=AuthPrompt.OWA-NT-Mail.Sharing.DirectLink.Copy&instantedit=1&wopicomplete=1&wdredirectionreason=Unified_SingleFlush" \l "_ftn1)

B.4.2.7 This will give the specific gravity of the alcohol liquid in air at 20±0.5°C

B.4.2.8 Record the temperature to the nearest 0.2°C

*Determine the ethanol content in % v/v of the spirit from the AOAC table of conversion.*

**B.4.3 Determination of ethanol content using densitometer method**

B.4.3.1 Activate the required method using the `method’ soft key.

B.4.3.2. Ensure that the measuring cell is clean and dry.

B.4.3.3 Draw the sample using a syringe ensuring no bubble are trapped in the syringe.

B.4.3.4 Fill the sample into the measuring cell by pushing the plunger of the syringe slowly and continuously until liquid comes out of the other side of the adapter.

B.4.3.5 Ensure that there are no gas bubbles in the measuring cell.

B.4.3.6 Record the results when the meter indicates that the measuring conditions are valid.

B.4.3.7 Fill in the next sample or clean and dry the measuring cell in-between, if necessary.

B.4.3.8 The result recorded from the meter should be multiplied by the dilution factor of two to get the ethanol content of the sample

[[1]](https://euc-word-edit.officeapps.live.com/we/wordeditorframe.aspx?ui=en-US&rs=en-US&wopisrc=https%3A%2F%2Fkebs770-my.sharepoint.com%2Fpersonal%2Fkitumf_kebs_org%2F_vti_bin%2Fwopi.ashx%2Ffiles%2Fb5040ef520bb421780bb56024ce3dff6&wdenableroaming=1&mscc=1&wdodb=1&hid=16373BA1-906F-9000-6004-12412042CAFA.0&uih=sharepointcom&wdlcid=en-US&jsapi=1&jsapiver=v2&corrid=f3c3211e-f448-d52b-9108-debab75b64f3&usid=f3c3211e-f448-d52b-9108-debab75b64f3&newsession=1&sftc=1&uihit=docaspx&muv=1&cac=1&sams=1&mtf=1&sfp=1&sdp=1&hch=1&hwfh=1&dchat=1&sc=%7B%22pmo%22%3A%22https%3A%2F%2Fkebs770-my.sharepoint.com%22%2C%22pmshare%22%3Atrue%7D&ctp=LeastProtected&rct=Normal&wdorigin=AuthPrompt.OWA-NT-Mail.Sharing.DirectLink.Copy&instantedit=1&wopicomplete=1&wdredirectionreason=Unified_SingleFlush#_ftnref1) When cooling the distillate and the distilled water in a volumetric flask in the absence of a cooler and/or an air conditioned room, top up to the mark with distilled water and cool the contents of the volumetric flask in a refrigerator or in ice and then bring the temperature to 20°C using the analyst’s hand, before weighing.

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**ANNEX C**

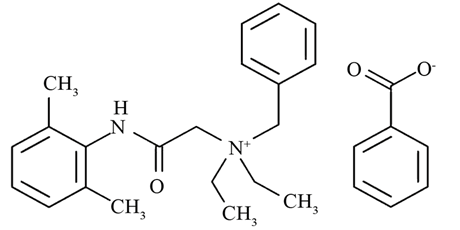
(Normative)

## 

**DETERMINATION OF DENATONIUM BENZOATE IN DENATURED ETHANOL USING**  **HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC-UV) METHOD**

**C.1 PRINCIPLE**

This standard method is used for the determination of denatonium benzoate (DB) in completely denatured alcohol (CDA) formulations using HPLC equipped with ultraviolet (UV) detection at a wavelength of 210 nm. The chemical structure of denatonium benzoate is shown in Fig.1 below. The samples are directly injected into the HPLC system after membrane filtration, if necessary. The working range for quantitative determination of DB is 5 mg/L to 200.0 mg/L.



**C.2 Reagents**

All reagents must be of HPLC grade or higher purity.

1. Denatonium benzoate (CAS: 3734-33-6), purity ≥ 99%. Handle with care.
2. Absolute Ethanol ≥ 96% by volume.
3. Sodium chloride (NaCl), extra pure
4. Acetonitrile, HPLC grade
5. Water, HPLC grade
6. Sodium chloride solution (0.2%). Weigh 0.4 g of sodium chloride in a weighing bottle and dissolve it in a beaker with 200 mL of HPLC grade water.
7. Mobile phase**:** Prepare in a 1000 mL volumetric flask by mixing sodium chloride solution in C with 800 mL of HPLC grade acetonitrile.

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## **C.3** **Apparatus**

1. HPLC system equipped with:
2. Analytical column: HPLC columns (C18 / C8), LiChrospher® 100 CN (5 µm) in LiChroCART® or equivalent.
3. 250-4 guard column
4. Thermostat column compartment
5. Diode array detector (DAD) or UV detector, capable of attaining 210 nm.
6. Electronic densitometer: used only to measure the density of the DB.
7. Analytical balance with a precision of 0.1 mg
8. 10 mL, 200 mL, and 1000 mL amber volumetric flasks.
9. Weighing bottle
10. Gas-tight syringes, 1 mL
11. 0.45 µm cellulose membrane syringe filters
12. 250 mL glass beaker
13. Wash bottle.
14. 1000 mL graduated measuring cylinder.

## **C.4** **PERFOMANCE**

**C.4.1Standard solutions**

## C. 4.1.1 **Preparation of the stock solution (1000 mg DB/L)**

Weigh, recording the exact weight, 0.1 g of denatonium benzoate (DB) into a 10 mL beaker and dissolve it in absolute ethanol. Mix gently and transfer to 100 mL amber volumetric flask.

Measure the mass of this solution on an analytical balance and the density at 20ºC with an electronic densitometer.

Calculate the concentration of the stock solution as follows:

**[𝐷𝐵, 𝑚𝑔/𝐿] 𝑠𝑡𝑜𝑐𝑘 = (𝐷𝐵 𝑚𝑎𝑠𝑠 (𝑔) 𝑥 106)/(𝑠𝑡𝑜𝑐𝑘 𝑠𝑜𝑙𝑢𝑡𝑖𝑜𝑛 𝑚𝑎𝑠𝑠 (𝑔))**

**𝑠𝑡𝑜𝑐𝑘 𝑠𝑜𝑙𝑢𝑡𝑖𝑜𝑛 𝑑𝑒𝑛𝑠𝑖𝑡𝑦 (𝑔/mL)**

## C.4.1.2 **Preparation of Calibration Standard Solutions**

Prepare the calibration standard solutions by serial dilution of the stock solution to the desired concentration levels between 5 mg/L to 200 mg/L.

## C.4.1.3 **Sample preparation.**

* + - 1. Weigh an empty 10 mL amber volumetric flask (6.2.4) and record the mass (VF1).
      2. Draw about 0.5 mL of the sample using a gas-tight syringe (6.2.6). Wipe the gas-tight syringe with lint-free paper towel and weigh on an analytical balance. Record the mass (M1).
      3. Transfer the sample into the pre-weighed volumetric flask and weigh the empty gas-tight syringe. Record the mass (M2).

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* + - 1. Top the volumetric flask containing the sample to the mark with the ethanol 96% (6.1.2). Weigh the volumetric flask with its contents and record the mass (VF2).
      2. Transfer sample solution into amber autosampler vials and perform HPLC determination within 24 hours of preparing the sample.

## **C.4.2** **Environmental control**

The analysis should be carried out in a well-ventilated air-conditioned room maintained at 20±2ºC since the retention time of the peaks fluctuates with temperature changes.

## **C.4.3** **Calibration**

The equipment used in this test shall be well calibrated. Periodic intermediate checks and general maintenance of the analytical balance (6.2.2) shall be done as per schedule.

## **C.4.4** **Quality control**

C.4.1 In daily, before the first measurement one of the calibration solutions, or a separately prepared reference solution or a reference material, is injected for performing QC verification. If the results are within ± 5 % of their theoretical values, analysis may proceed. If not, an investigation should be made to find the cause of the inaccuracy and remedial action taken as appropriate (i.e. new calibration curve).

C.4.2 The linearity (**R**) of the calibration curve should be above **0.999XX**. If the linearity is below the acceptable limit, repeat the calibration exercise

## **C.5.0** **PROCEDURE**

**C.5.1 High Performance Liquid Chromatography Setup**

Degas mobile phase solvents in a sonicator for 10 minutes and then connect solvent reservoirs to instrument. Turn on the instrument and load the method of test in the software. Download the method into the instrument. Allow software to connect to instrument and then open the pump valve. Press the purge button to prime the pump before use. Once purging is complete, close the valve and check if pressure is building up. Check for any air bubbles in the plumbing and purge the system if necessary. Install the appropriate column and check for any leaks. If leaks are detected, tighten the connectors. Allow the equipment to run for 20 min to equilibrate, with a different solvent other than the mobile phase flowing through; in this case the solvent is Methanol. After the equipment has stabilized, switch off the pump and switch to the mobile phase and allow the equipment to run for about 10 minutes. Ensure that the oven temperature of 25°C has been attained and any signals arising from the noise and drift of the equipment have been reduced. Adjust the flow rate of the mobile phase to 1.2 mL/min, set runtime at 10 minutes, the pump mode to isocratic mode, the lamp to D2, and detector wavelength set at 210 nm.

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**DKS 2041: 2024**

## **C.5.2** **HPLC Calibration Curve.**

To obtain a calibration curve, inject 1 µL of calibration standard solutions starting with the blank and then standards starting with the lowest concentration. Record the peak areas/heights for each corresponding concentration level. Ensure at least five replicate injections are made for each calibration standard. Prepare the calibration curve by plotting the peak areas/heights against DB concentration, in mg/L. Ensure that the results meet the stipulated acceptance criteria.

## **C.5.3** **HPLC Determination**

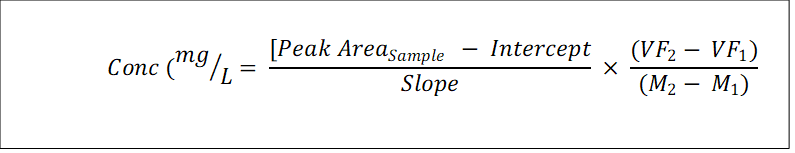
Inject 1 µL of the samples and record the peak area/heights. Using the peak areas/heights of the sample calculate the concentration of the sample using the Microsoft® Excel spreadsheet calibration curve (8.2). Ensure that at least three injections are made, and the average is reported as the result if it passes the QC checks stipulated in clause 7.4.2.

## **C.6.0** **RESULTS**

**C.6.1 Determination of denatonium benzoate content**

Denatonium benzoate content is determined using the following formula:

Where:



VF1 = mass in grams of empty volumetric flask

VF2 = mass in grams of volumetric flask with sample solution M1 = mass in grams of empty gas-tight syringe

M2 = mass in grams of gas-tight syringe with sample.

## **C.6.2** **Expression of Results**

Levels ³ 10 mg/L but < 100 mg/L to the nearest three significant figures

Levels ³ 100 mg/L to the nearest whole number

Levels > 0.1 mg/L < 10 mg/L to the nearest two significant figures.

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