BUREAU OF INDIAN STANDARDS

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Draft AMENDMENT NO. 1 OCTOBER 2022
TO
IS 17117 : 2019 HAIR SHAMPOO FOR BABIES — SPECIFICATION

(Page 2, Table 1, *Clause* 3.4 and E-5.1)—Substitute the existing Table 1 with the below table -

Sr. no. (1)	Characteristic (2)	Requirement (3)	Method of Test, ref to Annex/IS (4)
(i)	Active surfactant content, percent by mass, Min (Either of the following to be referred)	2 %	
	(a) Active detergent content, as SLES		Annex A
	(b) Active non-ionic and/ or anionic detergent content		Annex B
	(c) Milder surfactants - Glycinates and / or betaines		Annex H: Method for Glycinates and Betaines as per Annex given
(ii)	Non-volatile alcohol soluble matter, percent by mass, Min	4	Annex C
(iii)	pH at 27 ± 2ºC	4.0 – 7.5	Annex D
(iv)	Foam height of two percent solution, mm, Min	100	Annex E
(v)	Heavy metals (as Pb), parts per million, Max	20	Annex F
(vi)	Arsenic (as As2O3), parts per million, Max	2	Annex G or IS 16913 or IS 17495
(vii)	Microbial content/limit:		
	(a) Total microbial count, CFU/ml, Max	100	IS 14648
	(b) Yeast and mould count, CFU/ml, Max	100	IS 14648
	(c) Escherichia coli, per gram or per ml	Absent	IS 14648
	(d) Pseudomonas aeruginosa, per gram or per ml	Absent	IS 14648
	(e) Staphylococcus aureus, per gram or per ml	Absent	IS 14648

	(f) Candida albicans, per g or per ml	Absent	IS 14648
(viii)	Formaldehyde[a)], max ppm	10	Annex I (UV-vis) or Annex J (HPLC) or Annex K (GC HS)

Note – For Formaldehyde content UV-Vis method will be considered referee method in case of dispute, unless otherwise justified.

Insert the following new annexes for Method for Glycinates and Betaines, Formaldehyde estimation:

ANNEX H

[*Table* 1, *Sl No. i (c)*]

DETERMINATION OF MILD SURFACTANTS (GLYCINATES AND BETAINES) FROM BABY SHAMPOO

H-1 GENERAL

This method is suitable for the quantification of alkyl amidopropyl betaine and alkyl glycinates in baby shampoo using reversed-phase HPLC with UV detection. The samples are dissolved in methanol, filtered and analyzed by reversed-phase HPLC using a polar-embedded C18 column. The concentration of surfactant is determined using a UV detector and a three-point calibration curve. Typically, only the response from the C12 component peak of the surfactant is used for quantifying the analyte of interest.

H-2 APPARATUS

H-2.1 Analytical Balance with precision of ±0.05mg

H-2.2 Magnetic stirrer

H-2.3 Volumetric flasks, 50 & 100 mL

H-2.4 Volumetric pipets, Class A,1, 5 & 10 mL

H-2.5 Beaker, 1500 mL

H-2.6 Graduated Cylinder, 1000 mL

H-2.7 PTFE syringe filters, 0.45 µm, (Waters Corp P/N WAT200500 or equivalent)

H-2.8 Nylon solvent filters, 0.45µm, (Waters Corp P/N WAT200532 or equivalent)

H-3 REAGENTS

H-3.1 Acetonitrile, HPLC grade (**Caution:** Harmful, Flammable)

H-3.2 Methanol, HPLC grade (**Caution:** Toxic, Flammable)

H-3.3 Phosphoric Acid, 85% (**Caution:** Corrosive)

H-3.4 Raw Material Reference Standard (Prefer to use the one added in the formulation. Manufacturer can be contacted to provide respective reference standard)
Examples of Glycinate and Betaine commonly used are mentioned below

a. Sodium Cocoyl Glycinate - CAS 90387-74-9
b. Coco Amido Propyl Betaine - CAS 61789-40-0
c.

H-3.5 Water, HPLC grade or better

H-4 PREPARATION OF 0.1% PHOSPHORIC ACID MOBILE PHASE

H-4.1 Using a 1000 mL graduated cylinder, measure 1,000 mL of water and transfer to a mobile phase bottle.

H–4.2 Using a Class A volumetric pipet, transfer 1 mL of phosphoric acid to the mobile phase bottle.

H–4.3 Mix well and filter through a Nylon solvent filter.

H-5 PRIMARY STANDARD PREPARATION (1.0 mg/mL)

H-5.1 Correcting for purity, weigh 0.10 ± 0.01g of each surfactant into a 100 mL volumetric flask.

H-5.2 Dilute to volume with methanol and mix well.

H-6 WORKING STANDARDS (0.04, 0.20 & 0.40 mg/mL)

H-6.1 Pipet 1, 5 and 10 mL aliquots of the primary reference standard (3.2.2) into three separate 25 mL volumetric flasks.

> NOTE – Alternatively, only one standard to be prepared i.e., 0.20 mg/ml. The surfactant calculation can be done against the average area of replicate standards. When tested as per H-4.0

H-6.2 Dilute each flask to volume with methanol and mix well.

H-6.3 Filter each standard solution through a 0.45 µm PTFE syringe filter into an autosampler vial.

H-7 SAMPLE PREPARATION (0.7 – 6.7% SURFACTANT)

H-7.1 Weigh 0.15 ± 0.01 g of sample into a 25 mL volumetric flask.

> NOTE: Sample weight can be adjusted based on the concentration of surfactant used in the formula to match the concentration of standard and sample.

H-7.2 Add approximately 15 mL of methanol and mix using a stir bar for a minimum of 15 minutes to disperse the sample.

H-7.3 Dilute sample to volume with methanol.

H-7.4 Filter the sample solution through a 0.45 µm PTFE syringe filter into an autosampler vial.

H-8 PROCEDURE

H-8.1 Inject 5 replicates of the mid-point calibration standard onto the HPLC to establish system suitability

H-8.2 Inject each sample with an appropriate number of mobile phase and/or diluent blanks onto the HPLC.

H-8.3 Inject the mid-point calibration standard onto the HPLC at least once every 10 sample injections and again at the end of the run.

H-8.4 Prepare a calibration curve by plotting peak area of the C12 component of the respective surfactant versus concentration in mg/mL of the working standard solutions using a linear regression, which does not force the calibration line through zero.

> NOTE − Alternatively only one standard to be prepared i.e., 0.20 mg/mL. The surfactant calculation can be done against the average area of replicate standards.

H-9 HPLC CONDITIONS

Instrument:	Chromatograph equipped with an autosampler and UV detector or equivalent
Column:	Waters Xbridge C18, (4.6 x 150) mm, 3.5 µm or YMC pack ODS- A (150 x 4.6) mm, 3µm or equivalent
Mobile Phase A:	0.1% Phosphoric Acid in Water
Mobile Phase B:	100% Acetonitrile
Flow Rate:	1.5 mL/min
Detection:	UV @ 200nm
Injection:	10 µL
Temperature:	40^{o}C
Run Time:	14 minutes

GRADIENT TABLE

Time (min)	Mobile Phase A 0.1% H_3PO_4 in Water	Mobile Phase B 100% Acetonitrile
0.0	50	50
7.0	40	60
10.0	40	60

11.0	50	50
14.0	50	50

NOTE - The above gradient table may be modified to ensure specificity and resolution is achieved.

The retention times of the standards are:

Sample No	Analyte	Xbridge C18 column Retention Time (min)	YMC pack ODS column Retention Time (min)
1	C12- Betaine	2.19	2.67
2	C12-Glycinate	5.36	7.50

NOTE – the retention times are given for reference and may vary with change in equipment.

H-10 CALCULATION

Determine the weight percent of the surfactant from the calibration curve as follows.

$$Weight\% = \frac{(Peak\ Area\ of\ Sample - Intercept\ of\ Calibration\ Curve)(Final\ Sample\ Volume\ in\ mL)}{(Slope\ of\ Calibration\ Curve)(Sample\ Weight\ in\ mg)}\ x\ 100\%$$

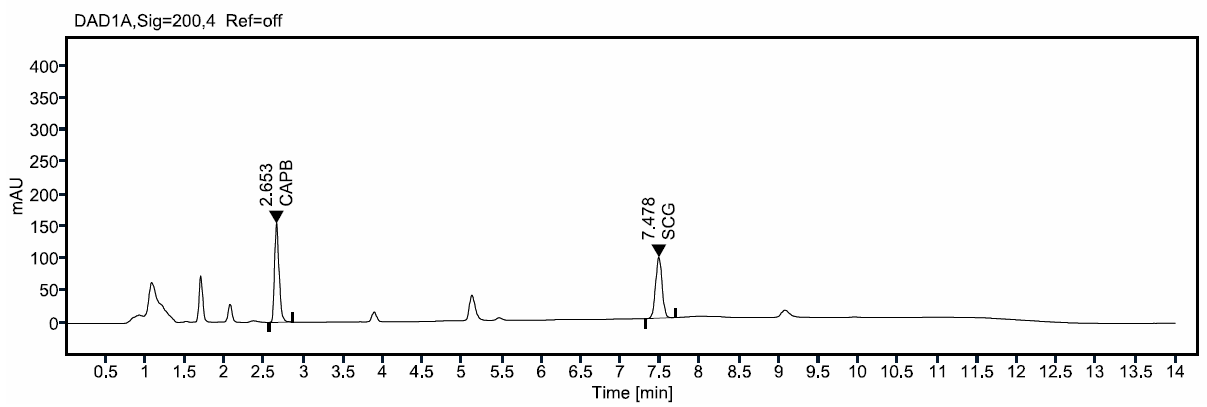
Note: Calculations based solely on the C12 peak assume equivalent chain length distributions of the raw material reference standard and the raw material ingredient in the product.

Determination of the weight percent of the surfactant when single standard used in the analysis:

$$\%\text{Content} = \frac{Areas\ of\ sample * Dilution\ Factor\ of\ Standard * Purity\ of\ standard}{Average\ Area\ of\ Standard * Dilution\ Factor\ of\ Sample}$$

$$\text{Dilution Factor of Standard/Sample} = \frac{Weight\ of\ Standard\ or\ Sample}{Dilution}$$

H-11 ANNEXURE



Representative Chromatogram of a 0.20 mg/mL coco amido propyl betaine and sodium cocoyl glycinate mixed reference standard on YMC pack ODS column.

ANNEX I

[*Table* 1, *Sl No. (viii)]*

DETERMINATION OF FORMALDEHYDE BY UV-VIS METHOD

Subject − Determination of Formaldehyde by UV-Vis Spectroscopy using derivatization with Acetyl Acetone solution

1 Scope and Purpose

The purpose of this method is determination of Formaldehyde in Johnson's shampoo formulations by UV-VIS Spectroscopy.

2 Safety precautions

The analyst should follow general laboratory practices and use appropriate personal protective equipment. The analyst should read Material Data Sheets (MSDS) of Formaldehyde before handling.

3 Principle of method

Formaldehyde is reacted with Acetyl Acetone in an Ammonium Acetate buffer system to produce a yellow colour derivative. The absorbance of the yellow solution is determined using an Ultraviolet-Visible Spectrophotometer and quantitation is achieved by comparison with a standard.

4 Fundamental equations

None.

5 Interferences

Any extraneous component having a significant absorbance at 412nm will interfere.

6 EQUIPMENT

6.1 UV-Visible Spectrophotometer

6.2 1-cm quartz or optical glass cuvette

6.3 Water Bath

6.4 50ml centrifuge tubes

6.5 15ml Test tube with cap

6.6 1000 µl micro pipet

6.7 100 mL and 50mL volumetric flasks

6.8 5mL, 10mL, 20mL volumetric pipettes

7. REAGENTS

7.1 Formaldehyde Reference Standard, Purity ≥ 37%, - e.g. Sigma-Aldrich

7.2 Acetyl Acetone (2, 4-Pentanedione), J. T. Baker Cat# JTS926-6 or equivalent

7.3 Acetic Acid, Glacial, Reagent Grade

7.4 Ammonium Acetate, Reagent Grade

7.5 Sodium Sulfate, Reagent Grade

7.6 Isopropanol (IPA), Reagent Grade

7.7 Purified Water or equivalent

7.8 Acetyl Acetone Working Solution: Weigh 37.5gm of Ammonium Acetate and

quantitatively transfer to 250ml volumetric flask with purified water. Pipet 0.75ml of Acetic Acid and 0.5ml of Acetyl Acetone into the flask. Dissolve and dilute to the mark with purified water. Mix well, protect from light. This solution should be made fresh for use.

7.9 25% Sodium Sulfate Solution: 125gm of Sodium Sulfate transfer gradually to 500 ml volumetric flask which is half filled with purified water, keep mixing with the stirrer. Dissolve and dilute to the mark with purified water, mix well until completely dissolved.

8. Standard Preparation

8.1 Standard stock solution: (Formaldehyde 1000 µg/mL)

Accurately weigh 0.270±0.01g of Formaldehyde standard (considering 37%w/v Formaldehyde standard) into a 100 mL volumetric flask. Dilute to volume with water and mix well.

8.2 Intermediate standard solution (Formaldehyde 100 µg/mL)

Pipette 10.0 mL of standard stock solution into a 100mL volumetric flask. Dilute to volume with water and mix well.

8.3 Working standard Solution: (Formaldehyde 5 µg/mL)

Pipette 5.0 mL of intermediate standard solution into a 100mL volumetric flask. Dilute to volume with water and mix well.

8.4 Prepare another standard solution called as the "Check Standard" by repeating steps 8.1 through 8.3.

9. Sample preparation

Weigh accurately 1.0gm ±0.001 into 50 ml centrifuge tube, pipet 20ml of Sodium Sulfate solution and 20 ml of purified water. Shake well, then keep the sample in a 40ºC bath for 30 minutes. Centrifuge the sample tube for 20 minutes at 3500 rpm. Use supernatant sample solution for derivatization as per 10.1.

10 Procedure

NOTE: The blank, standard and sample solution are placed in the water bath at 40ºC and then allowed to react at the same time.

10.1 Pipet 5ml of 5ppm standard (Working standard solution & Check standard Solution given in 8.3 and 8.4) and sample (9) into each 15 ml test tube with cap.

10.2 Prepare blank by pipetting 5 ml of water (7.7) into a tube.

10.3 Pipet 5 ml of Acetyl Acetone working solution (7.8) into each of the tubes, cap them and mix well.

10.4 Equilibrate the tubes in a 40ºC water bath for 30 minutes.

10.5 Remove the tubes from the bath, cool to room temperature and add 1ml of Isopropanol to sample tube. If this solution is clear, then add same amount of Isopropanol to blank, standards and additional samples, if any.

10.6 If sample is still hazy after 1 ml of IPA added to the sample, add 1 ml of IPA more to standard, sample and blank. Do not add more than 3 ml of total IPA. Mix the solution gently. The solution in each tube must be clear.

NOTE: For sample that are highly coloured, the sample can be analysed without acetyl acetone to check the possible interference from the sample matrix or as appropriate

11 OPERATING CONDITIONS

11.1 Turn on the lamp of the UV/Visible Spectrophotometer.

11.2 Warm up the lamp for at least 15 minutes.

11.3 Set the instrument at a fixed wavelength of 412 nm.

11.4 Autozero the instrument with blank solution.

11.5 Use the blank solution as reference for all further measurements.

11.6 Rinse the cuvette carefully with the Working Standard Solution and fill it to the appropriate level.

11.7 Insert the cuvette into the cuvette holder. The cuvette needs to be inserted the same way each time during the readings.

11.8 Read the absorbance for the Working Standard Solution

11.9 Read absorbance for the Check Standard Solution. Calculate the percentage of check standard conformity. The percentage of check standard conformity must agree within 97.0 to 103.0%.

NOTE: If the percentage of check standard conformity does not agree within 97.0 to 103.0%, the third standard should be prepared according to section 8 (Standard Preparation). Read the absorbance for the third standard. The percentage of check standard conformity must agree within 97.0 to 103.0% considering the third standard.

11.10 Read the absorbance for the sample solution.

11.11 Read the absorbance of the Reference Standard once after maximum of ten sample readings and at the end of the run.

11.12 The % RSD for absorbance of the complete set of the refence standard measurements should not exceed 4.0 %

11.13 Use the average absorbance of all the working standard solutions (STD**)** for sample calculation.

12. CALCULATION AND REPORTING

12.1 Check Standard Conformity:

$$\% \text{ Check standard conformity} = \frac{A2 \times W1 \times 100}{A1 \times W2}$$

Where

A1 = Absorbance reading of working standard standard
A2 = Absorbance reading of check standard
W1 = Weight of working standard in gm
W2 = Weight of check standard in gm

12.2 The Concentration of Formaldehyde In Sample:

$$\text{ppm of formaldehyde} = \frac{\text{Absorbance of sample} \times \text{ppm of STD} \times 40}{\text{Average absorbance of STD} \times \text{Weight of Sample}}$$

Where:

40 = dilution factor
Weight of Sample = Weight of sample in gm
STD = Working standard solution

$$\text{ppm of STD} = \frac{X \text{ gm} \times 10 \times 5 \times \% \text{ Purity of Formaldehyde} \times 1000000}{100 \times 100 \times 100 \times 100}$$

$$= X \text{ gm} \times \% \text{ Purity of Formaldehyde} \times 0.5$$

ANNEX J
[*Table* 1, *Sl No. (viii)]*

DETERMINATION OF FORMALDEHYDE BY HPLC METHOD

1 OBJECTIVE OF THE METHOD

Quantification of formaldehyde in finished products by derivation with 2,4-dinitrophenylhydrazine (2,4-DNP) then reverse phase assay on a column type C18.

The quantification of formaldehyde is recommended by dosed additions. Indeed, accuracy and precision are better than quantification by external calibration. With dosed additions, all samples are derived under the same conditions by being integrated into the same bypass reactor. Screening of aldehydes in finished products.

2 PRINCIPLE

Reverse phase HPLC assay after reaction medium derivation of FORMALDEHYDE by DNPH by UV/Visible detection. Qualitative screening of aldehydes in finished products by this same methodology.

3 MATERIALS REQUIRED

3.1 Hardware

3.1.1 HPLC with diode array detector or equivalent.

3.1.2 HPLC C18 column; 250x4.6 5µm

3.1.3 pH meter; Mettler Toledo or equivalent.

3.1.4 Thermomixer Comfort Eppendorf;

3.1.5 support flacon 15ml. Falcon 15 ml

3.1.6 polypropylene reaction tube

3.1.7 Filter ACRODISC GHP 25mm 0.45*m;

3.2 Reagents and Scales

3.2.1 Formaldehyde in 16% aqueous solution (m/v) in 10 ml ampoule

3.2.2 2,4-DinitropphenylHydrazine 97% stabilized with 30% water

3.2.3 37% Hydrochloric Acid

3.2.4 Acetonitrile Gradient Grade

3.2.5 Water Quality HPLC, MILLI-Q System or equivalent.

4 METHODOLOGY

4.1 Preparation of reagents

SOLUTION DE DERIVATION (DNPH)

Preparation of a derivation solution based on DNPH at 1,5 g/l:

- Introduce 310mg of 97% DNPH in a 200ml volumetric flask.
- Complete with the gauge line with the

Acetonitrile

Gradient Grade. This solution is kept at 4 ° C away from light no more than two days.

4.2 Analytical conditions

Chromatographic conditions for quantification by external calibration

Track A: ACN B: MeOH

Track C: ACN D: Water quality HPLC

Injection of 20 µl.
Detection: (recording from 320 to 400 nm) 360 and 349 nm Column oven thermostated at 30 ° C.

Sample compartment at 6°C.

For information, at T0 the pressure is about 1650 psi

For the determination of formaldehyde

The analysis is first done in isocratic mode and then in a second time in gradient mode to eliminate all other unanalyzed components from finished products.

	Time	Flow	%A	%B	%C	%D	Curve
1	0.01	1.20	0.0	0.0	60.0	40.0	6
2	6.00	1.20	0.0	0.0	60.0	40.0	6
3	13.00	1.20	0.0	0.0	69.0	31.0	6
4	14.00	1.20	0.0	0.0	100.0	0.0	6
5	16.00	1.20	0.0	0.0	100.0	0.0	6
6	16.10	1.20	0.0	0.0	60.0	40.0	1
7	20.00	1.20	0.0	0.0	60.0	40.0	6

For the qualitative screening of aldehydes.
The analysis is done first in isocratic mode and then in a second time in gradient mode to eliminate all other unanalyzed components from the finished products.

	Time	Flow	%A	%B	%C	%D	Curve
1	0.01	1.20	0.0	0.0	60.0	40.0	6
2	6.00	1.20	0.0	0.0	60.0	40.0	6
3	13.00	1.20	0.0	0.0	69.0	31.0	6
4	14.00	1.20	0.0	0.0	100.0	0.0	6
5	30.00	1.20	0.0	0.0	100.0	0.0	6
6	31.00	1.20	0.0	0.0	60.0	40.0	6
7	40.00	1.20	0.0	0.0	60.0	40.0	6

4.3 Measurements, retention time

These retention times are given as an indication, under the conditions of the test.

HPLC Visible detection, λ=360 nm for: Formaldehyde
at 4.20 mn

4.4 Preparation of standards / Calibration range

4.4.1 *Preparation of standards*

Preparation for external calibration: Formaldehyde

4.4.2 *Range of the aldehyde*

To prepare the mix 3 mol stock solution and the SF2 daughter solution, a standard range is prepared in 20 ml vials, not forgetting to add in each vial 30µl of 37% hydrochloric acid for the diversion which must be done in an acidic medium.

	VolSM2 mix3mol (ml)	Vol SF2 mix3mol (ml)	HCl at 37% (ml)	Ampoules (ml)	**Concfinal formaldehyde (µg/mL)**
White	-	-	0.030	Qsp 20 mL H2O	**0.00**
STD 1	-	0.10	0.03	Qsp 20 mL	**0.200**

		0	0	H2O	
STD 2	-	0.200	0.030	Qsp 20 mL H2O	**0.400**
STD 3	-	0.500	0.030	Qsp 20 mL H2O	**1.00**
STD 4	-	1.000	0.030	Qsp 20 mL H2O	**2.00**
STD 5	0.050	-	0.030	Qsp 20 mL H2O	**10.0**
STD 6	0.100	-	0.030	Qsp 20 mL H2O	**20.0**
STD 7	0.200	-	0.030	Qsp 20 mL H2O	**40.0**

NOTE − It is essential to make a blank to estimate the amount of formaldehyde in the DNPH

4.4.3 *Derivation of the range by the 2,4-DNPH. (External calibration)*

Standards and white must be derived by the DNPH before being analyzed. The leads are made at 50 ° **C**. Before starting the preparation, it is important to program the Thermomixer(s) at the right temperature.

4.4.4 *Formaldehyde*

4.4.4.1 Prepare and label 8-15ml "Falcon" tubes

4.4.4.2 For each of 8 solutions of the standard range put 5ml in one of the 8 tubes "Falcon"

4.4.4.3 In each tube add 5 ml of the DNPH bypass solution.

4.4.4.4 Homogenize rapidly at the vortex

4.4.4.5 Place the reaction tubes in the already temperature-stabilized thermomixer

4.4.4.6 Cover the vials with aluminum foil. **The reaction must be to the dark**.

4.4.4.7 Program and start the appropriate cycle:

4.4.4.8 from 2H to 50°C with a stirring of 700 rpm

4.4.4.9 At the end of this time, filter on GHP ACRODISC filter 0.45µm in the injection vials.

4.4.4.10 The vials are kept away from light at 6°C in the cooling compartment of the HPLC system before injection.

4.4.4.11 20µl of each solution is injected to establish the calibration range. Vials must be kept away from light between 6 and 10°C

4.5 Preparation for external calibration:, Formaldehyde

4.5.1 In a 100ml volumetric flask, weigh about 200 mg of sample (PF or MP).

4.5.2 Add about 50 mL of water and then add 0.150ml of 37% hydrochloric acid

4.5.3 Compléter to the gauge line by WATER quality HPLC.

4.5.4 If the product is presumed to be highly concentrated, this solution may be diluted. This dilution should be done in water quality HPLC acidified by 30µl of 37% hydrochloric acid per 20ml of solution.

4.5.5 The derivation by the DNPH is then carried out in a "Falcon" tube as indicated below:

4.5.6 In a tube

- place 5 ml of the acidified solution PF or MP
- Then add 5 ml of the DNPH bypass solution

4.5.7 Homogenize rapidly at the vortex

4.5.8 Place the reaction tubes in the Thermomixer

4.5.9 Cover the vials with aluminum foil. . **The reaction must be to the dark**.

4.6 Program and start the appropriate cycle:
- **from 2H to 50 ° C with a stirring of 700 rpm**

4.6.1 At the end of this time, filter on GHP ACRODISC filter 0.45µm in the injection vials.

4.6.2 The vials are kept away from light at 6°C in the cooling compartment of the HPLC system before injection.

4.6.3 Perform 3 tests per sample.

4.6.4 Homogenize rapidly at the vortex

4.6.5 Place the reaction tubes in the Thermomixer

4.6.6 Cover the vials with aluminum foil: The reaction should be in the dark.
- Program and start the cycle of 30 minutes at 10 ° C with a stirring of 700 rpm.

4.6.7 At the end of this time, filter on GHP ACRODISC filter 0.45µm in the injection vials.

4.6.8 The vials are kept away from light at 6°C in the cooling compartment of the HPLC system before injection.

For external calibration: Glyoxylic acid, Acetaldehyde, Formaldehyde, Glyoxal

For Formaldehyde and acetaldehyde, detection is made at 360 nm. Standard settings allow for good integration. The calculation of the calibration line is carried out on the surface of the chromatographic peaks **in linear regression with a weighting in 1/X**.

Calculation taking into account the dilution of the samples:
Measure the surface area of the peaks corresponding to the formaldehyde, for the five samples prepared.
Draw a straight line by carrying on abscissa the concentrations du compound added in the mixture (matrix + standard) in µg / g and in ordinates the surfaces of the corresponding peak.
We obtain a line of the type: $y = Ax + B$

Injected formaldehyde content (µg/g):

$$C_i = \frac{B}{A}$$

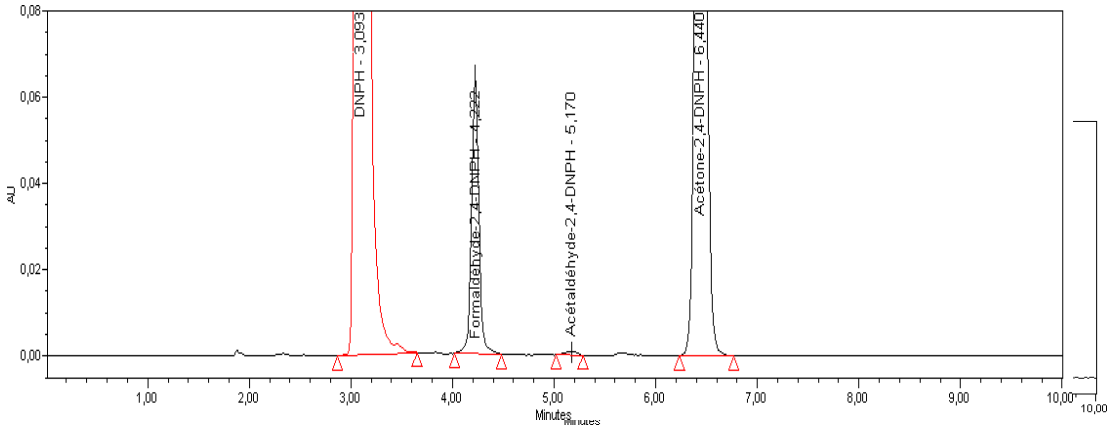
Formaldehyde concentration in the matrix (µg/g sample):

$C_{MT} = C_i \times \frac{M_M}{M_{PE}}$	*With:* - ***A*** *= Slope of the calibration line.* - ***B*** *= Intercept of the calibration line.* - ***Ci*** *= Concentration of* injected *formaldehyde (µg/g).* - ***CM*** *= Formaldehyde concentration in the matrix (µg/g sample).* - ***MT*** *= Total mass of the prepared control sample ECH0 (matrix + solvent) (g).* - ***MPE*** *= Mass of the test portion of the control matrix ECH0 (g).*

5 CHROMATOGRAMS AND SPECTRA: FORMALDEHYDE

Chromatogram of a white DNPH

Chromatogram of point 5 of the range



Spectrum of Formaldehyde-2,4-DNPH

ANNEX K

[*Table* 1, *Sl No. (viii)]*

DETERMINATION OF FORMALDEHYDE BY GC HS METHOD

1 ANALYSIS REQUIREMENT

1.1 Gas Chromatography: Shimadzu GC-2010 Plus with HS sampler or any other equivalent

1.2 GC column: DB-624 capillary column, 30m length, 0.32mm i.d, 1.8 µm Film thickness. (Part. No: -123-1334, Make: - Agilent) or any other equivalent

1.3 Working standard/ Reference standard: Formaldehyde solution 37% (Sigma Aldrich)

1.4 Sample Details: Shampoo

1.5 Chemical /Reagent Details:

Ethanol (AR)

p- Toluene sulphonic acid monohydrate (PTSA) (AR)

2 CHROMATOGRAPHIC CONDITION.

Column	:	DB-624 capillary column, 30m length, 0.32mm i.d, 1.8 µm Film thickness. (Part. No: -123-1334, Make: - Agilent) or any other equivalent
Detector	:	FID@300°C
Mobile phase	:	Nitrogen gas
Elution type	:	Temperature Gradient (Refer table no.2)
Flow rate	:	1.0 ml/minute
Diluent	:	1.5 % PTSA in Ethanol (AR grade)
GC cycle time	:	50 minutes
HS Oven temperature	:	70°C
Sample line temperature	:	110° C
Transfer line temperature	:	140°C
Shaking level	:	4
Pressurize gas pressure	:	100kPa
Split ratio	:	1:5
HS equilibration time	:	50min
Pressurization time	:	2.0min
Pressure equilibration time	:	0.1min

Load time	:	0.5min
Load equilibration time	:	0.1min
Injection time	:	1.0min
Needle flush time	:	10min

2.1 Column Oven Temperature Program

Rate/min	Temperature	Hold (min)
--	35°C	7
5°C/min	120°C	1
50°C/min	220°C	9

2.2 Preparation of 1% para-Toluene Sulphonic Acid in Ethanol: - Accurately weigh 7.5g of para-Toluene Sulphonic Acid monohydrate and dissolve in 500 ml absolute ethanol.

2.3 Standard Preparation (Stock solution): Accurately weigh 135mg of 37% Formaldehyde standard solution to a 50 ml volumetric flask, add about 30ml diluent, sonicate for 2mins. Make the volume up to the mark with diluent and mix well.

2.4 Working Standard solution: Pipette out 1 ml of the stock solution to a 100 ml volumetric flask and dilute up to the mark with diluent. Pipette out 5ml of the working standard solution into a standard GC-headspace vial and seal it with aluminum crimp cap with silicone septa.

2.5 Sample Preparation: Accurately weigh 250mg of sample to a standard 20ml GC-Headspace vial and add 5ml diluent. Seal the HS-vial with aluminum crimp cap with silicone septa.

2.6 System suitability

Inject the diluent as blank until a stable baseline is achieved. Inject the standard and again inject the blank to check the carryover.

Make six replicate injections of standard solution and check the % RSD of the area of standard. Continue the sample analysis once the following system suitability parameters are achieved.

Retention time for Formaldehyde	:	15minutes
Relative standard deviation (% RSD for 6 replicate injections)	:	NMT10.0%
Tailing Factor	:	NMT2.0
No. of Theoretical plates	:	NLT2000

3 Calculation:

FC = (((A1)/(A2))*((W2)/(50))*((1)/(100))*((5)/(W1))*(P)*(10000))

Where

A1-Average area of sample peak corresponding to standard peak
A2-Average area of standard peak

W1-Weight of sample (mg)

W2-Weight of standard (mg)

P-% purity of standard

FC=Formaldehyde content (ppm)