DETECTION OF BINDER (KYMENE RAW MATERIAL SOLUTION) IN CELLULOSE TISSUE

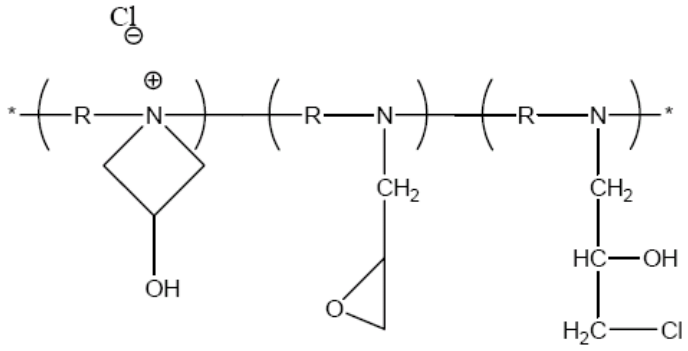
1. Scope and application

This method allows the detection of Kymene wet-strength agent in cellulose tissue samples, at a minimum level of 0.05% by weight (LOQ). Kymene is a water-soluble polymer by Hercules Incorporated based on Polyamide-Epichlorohydrin (PAE) resin.

2. Summary of method

Kymene (see typical structure in Fig.1), bonded to cellulose fibres, is released by acid hydrolysis. The adipic acid produced during the hydrolysis is derivatized with hot sodium methoxide to get dimethyl adipate. The adipate is extracted in hexane and then analyzed by gas chromatography coupled with mass spectrometry detector.

Fig.1



3. Reference

- EC Regulation No. 796/2002 amending the 2568/91 – Preparation Methods of methyl esters of fatty acids (Method B: Methylation by heating with sodium methoxide in methanol followed by esterification in acid room);
- "Development of a method for finding the Kymene in cellulose samples" - (M.Polidoro, Greenlab, 26/11/2010);
- Lab-Notebook L. D'Eugenio N.29, page 111-112.

4. Interferences

The risk of interferences is highly reduced because of the MS detection via specific dimethyl adipate m/z (see calculation section). However for each set of analyzed samples, we recommend to check potential environmental contamination following the procedure using the reagents only, without cellulose tissue.

5. Equipment

- Class A flask of 500mL
- Hot plate with water cooler
- Separator funnel of 1L
- Flasks of 100 mL A Class
- Micro-syringe 25ul
- Vial for 2 mL auto-sampler
- Analytical balance capable of weighing 0.0001 g.
- Rotavapor BUCHI R-114 with Waterbath B-480 or equivalent
- Gas chromatograph Thermo Scientific Trace GC Ultra, coupled with Mass Spectrometer Thermo Scientific DSQ II or equivalent
- Capillary Column Thermo TR-5MS L=30 mt, ID=0.25mm, FT=0.25µm or equivalent

6. Reagents and Solutions

- Ultrapure osmotic water
- GC grade methanol by Sigma-Aldrich cod. 34860
- N-hexane, for residual analysis by Fluka cod. 34484
- Hydrochloric acid 37% ACS grade by Sigma-Aldrich cod. 30721
- Concentrated sulphuric acid ACS grade by Sigma-Aldrich cod. 30743
- Dimethyl phthalate by Fluka cod. 36738
- Sodium metallic pure by Aldrich, cod. 13401
- Sodium Sulphate anhydrous ACS Sigma-Aldrich cod. 71960
- Hydrolizating solution: ultrapure water / HCl conc 37% in the ratio 4: 1 v/v
- Sodium methoxide 0,5 N: dissolve cautiously 12.5 g of sodium in 1 L of anhydrous methanol
- Sulphuric acid in methanol solution: dissolve very cautiously 50 mL of sulphuric acid conc. in 150 mL of methanol

- Phenolphthalein: 0,2 % in methanol
- Sodium hydroxide 6M solution
- Saturated of sodium chloride solution
- Internal Standard: solution of 100 mg/L of dimethyl phthalate in hexane
- Kymene Reference Standard (water solution) supplied by the customer

7. Test Procedure

Acid hydrolysis (kymene conversion in adipic acid)

Introduce into a 500 mL flask about 3.0000g of cellulose tissue reduced in size of about 4-6 cm^2 with scissors. Apply the water cooler and add 200 ml of hydrolyzing solution prepared by mixing 160 mL of osmotic water with 40 mL of HCl 37%. Heat at 200-250°C and boil it under reflux for 3 hours. Cool and add 5 drops of phenolphthalein. Add NaOH 6M (about 80-90 mL) to neutralize the solution (solution becomes darker), and then add 1 mL more to make sure that the pH is higher than 11. Evaporate the water by rotavapor under vacuum (about 300 mbar) and with a bath temperature of 95°C. Dry the residue putting the flask in the oven at 105°C for at least 2 hours.

Derivatization (methylation of adipic acid)

Wash down the cooler with two portions of 10 mL of sodium methoxide to eliminate water residual. Re-Connect the reaction flask with dry residue and introduce 150 ml of methanol solution 0.5 N of sodium methoxide. Bring to boil, making sure that all the material deposited on the walls end up in solution. Boil for 20 minutes, stirring occasionally to catch any residue on the walls of the flask. Remove the flask from the heat source, wait that the reflux ends up and introduce, through the cooler, sulphuric acid methanol solution until the solution becomes clearer, and then add 3 ml in excess. Boil for additional 20 minutes. Remove the heat source, add through the cooler 100 ml of saturated solution of sodium chloride and shake. Add another 300 mL of osmotic water to dissolve the saline residue that prevents the liquid-liquid extraction step.

Liquid-liquid extraction of dimethyl adipate

Unplug the cooler, stir again, transfer the content from reaction flask to a 1L separatory funnel and extract with 3 portions of hexane (100-100 and 50 mL). Transfer the extracts in a flask and add an amount of sodium sulphate anhydrous to .adsorb water residual. Transfer the extract into a 500 mL flask, rinse the sodium sulphate with two 10 ml portions of hexane. Concentrate on rotavapor bath (40°C with 300 mbar of vacuum) to about 50 mL. Bring to a 100 mL final volume of hexane in a flask. Transfer 1 mL to a vial auto-sampler, add 25µL of internal standard: 100 mg /L of dimethyl phthalate in ethyl acetate. Inject the gas chromatograph.

Instrumental conditions

Oven program: 50°C (1min) → 25.0°C/min → 300°C (6 min)

PTV Injector: PTV Splitless for 1.0min, Transfer Temp. 290°C, Transfer Rate 14.5 °C/sec,

Base Temp. 80°C, Split Flow 10mL/min

Carrier: Helium 1.5 mL/min constant flow

Transfer Line: 250°C

Injected volume: 1 µL

MS Detector: Full scan (50-200 amu), delay time 3.0min, Scan Rate 1000 amu/s

SIM Mass (dwell time 100ms, Width 1): 101,111,114,143 (adipate), 163 (int.std.)

8. Calculation

Calculation is based on Area ratio response value (formula 1) between dimethyl adipate (101 +111 + 114 + 143 m/z) and Internal Standard (163 m/z).

Since we have to demonstrate that the level in the cellulose samples is below 0.05 % by weight, we compare the Area ratio response value of cellulose blank sample vs. a cellulose sample spiked with a fixed and representative amount of wet-strength agent (blank samples + 0.05% by w/w of Kymene standard solution raw material)

Formula 1 $$\text{Area Ratio} = \frac{AreaDimethylAdipate}{AreaISTD}$$

In case of Positive value results use formula 2

$$\%\ \text{of Kymene raw material (solution)} = \frac{AreaDimethylAdipate}{AreaISTD} \times$$

Please note that to get the real % of Kymene raw material in tissue; it is necessary to adjust the calculation with the exact Kymene concentration in water solution.

9. Validation Summary

Since the method is just to discriminate wet strength agent presence in cellulose tissue samples below 0.05% we have checked the following validation parameters.

1. LOQ

   0.05% is the LOQ based on the value we got on cellulose spiked sample that showed a standard deviation of area ratio signal at least three times higher (success criteria) than the standard deviation of blank cellulose sample.

2. Specificity

   Environmental contamination has been checked on blank reagents in six replicate (table1)

   Table 1

Reagents check	Area ratio Value
AVERAGE (6 replicates)	0,183
DEV. ST.	0,0352
RSD. %	19

   Blank cellulose tissue interferences (table 2) have been checked on six bases replicate. It showed an area ratio signal at least three times lower than blank cellulose tissue spiked with 0, 05% of Kymene area ratio signal (table 3)

   Table 2

Blank cellulose tissue (kymene-free)	Area ratio Value
AVERAGE (6 replicates)	4,99
DEV. ST.	0,972
RSD. %	19

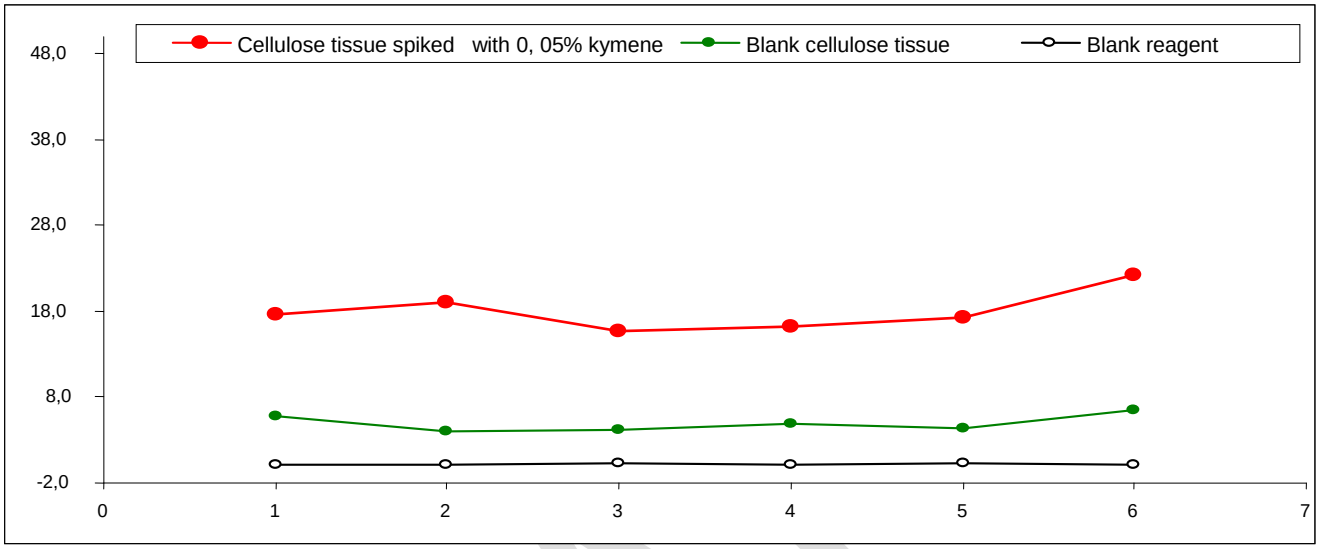
3. Repeatability

   The repeatability of the method has been evaluated on 6 bases replicates of blank cellulose tissue spiked with 0, 05% kymene by weight). Success criteria was to get a RSD < 20%.
   Below table (average data) and graph that summarized the results we got for method repeatability check:

   Table 3

Blank cellulose tissue + Kymene 0.05%	Area ratio Value
AVERAGE (6 replicates)	18,1
DEV. ST.	2,38
RSD %	13

Graph below summarize the signal area ratio of specificity and repeatability check.



Area ratio signal of blank cellulose tissue + 0,05% of Kymene, is above three times the standard deviation value of blank cellulose tissue (tissue Kymene Free)

Likewise blank cellulose tissue showed a standard deviation of area ratio signal three times higher than blank reagent one.

Attachment

Below a picture with the GCMS profile of a cellulose sample containing Kymene binder with the following information:

1. TIC profile
2. SIM profile of Dimethyl adipate (Tr= 5.73, SIM m/z= 101, 111, 114 and 143),
3. SIM profile of Internal standard ((Tr=7.00, SIM m/z= 163)
4. Mass Spectrum of Dimethyl adipate