DETERMINATION OF FREE Methoprene in CS FORMULATIONS

SCOPE

The method is intended only for the determination of free methoprene in CS formulations.

OUTLINE OF METHOD

A known quantity of the capsule suspension is transferred to a glass bottle and then subjected to a process of rotary evaporating extraction with a specified amount of n-hexane containing an internal standard. After mixing/extraction for a specified period, the content of free methoprene in the n-hexane layer is determined by GC-FID.

REAGENTS

Water conforming to ASTM Type II.
APPARATUS
250 ml Glass flask glass
Rotary evaporator
Timer capable of measuring to the nearest second

(1) Extraction of free methoprene

Prepare solutions in duplicate for each sample. Weigh approximately 20 g (to the nearest 0.1mg) of the sample into a 250 mL glass flask with a stopper, pipette 5 mL of water, cap and shake well until completely dispersed and add 100 mL of the n-hexane internal standard solution prepared. Place the above solution on a rotary evaporator at atmospheric pressure at ambient temperature for 5 minutes at 70 rpm. The bottle is removed, placed vertically on a flat surface and left to stand for 1 minute. Take 1 mL of the top solution (taking care not to extract any precipitate containing n-hexane) and place in an autosampler vial (solutions S_A and S_B).

(2) Determination of free methoprene

OUTLINE OF METHOD
The amount of free methoprene in the n-hexane layer is determined by capillary gas chromatography.

REAGENTS
Methoprene standard of known purity
n-hexane HPLC grade. n-hexane is harmful by inhalation and prolonged exposure may cause serious health damage. Always handle and use this material, with the appropriate PPE within a well-ventilated area.
Eicosane internal standard. Must not contain impurities with the same retention time as methoprene.
Internal standard solution. Dissolve Eicosane (1000mg) in n-hexane (1000ml).

Calibration solution. Weigh in duplicate (to the nearest 0.1 mg) 100 mg (*s* mg) of Methoprene standard into a volumetric flask (100ml) and make up to volume with internal standard solution, Mix thoroughly (calibration solutions C_A and C_B).

APPARATUS

Gas chromatograph capable of operating within the range from 100 to 300 °C, equipped with a flame ionisation detector and a split/splitless injector
Capillary column 30 m × 0.25 mm (i.d.), coated with 100 % bonded and cross-linked dimethylpolysiloxane, film thickness 0.25 µm
Automatic digital integrator or *chromatography data system* compatible with the gas chromatograph

PROCEDURE

(a) Chromatographic conditions (typical):

Column Fused silica, 30 m × 0.25 mm (i.d.) coated with 100 % chemically bonded and cross-linked dimethylpolysiloxane, film thickness 0.25 µm

Injection system

Injector Split injection. Check the condition of the liner prior to analysis.

Split ratio: 100:1

Split flow: 140 ml/min

Detector flame ionisation

Temperatures

Injector: 250 °C

Detector: 300 °C

Oven program initial temperature: 170 °C
program rate: 10 °C/min
final temperature: 240 °C (5 min)

Injection volume: 1 µl

Flow rate

Nitrogen (carrier gas) 25 ml/min (pressure: 150 kPa)

Retention times methoprene: about 7.3 min
eicosane: about 6.4min

(b) Linearity check. Before conducting the analysis check the linearity of the detector response by injecting 1 µl of solutions with methoprene concentrations 0.5, 1 and 2 times that of the calibration solution.

(c) System equilibration. Prepare two calibration solutions. Inject 1 µl portions of the first one until the response factors obtained from two consecutive injections differ by less than 1.0 %. Then inject a 1 µl portion of the second solution. The response factor for this solution should not deviate by more than 1.0 % from that for the first calibration solution, otherwise prepare new calibration solutions.

(d) Determination. Inject in duplicate 1 µl portions of each sample solution (S_A and S_B) bracketing them by injections of the calibration solutions (C_A and C_B) as follows:
C_A, S_A1, S_A2, C_B, S_B1, S_B2, C_A ... etc.
Measure the relevant peak areas.

(e) Calculation. Calculate the response factors (f_i.) for the pair of calibration injections which bracket the sample injections S_A1, S_A2 etc. and calculate the mean response factor *f*.

$$f_i = \frac{I_r \cdot s \cdot P}{H_s}$$

Methoprene content =

$$\frac{f \cdot H_w}{I_q \cdot w}$$ g/kg

where:

f_i = individual response factor

f = mean response factor

H_s = peak area of methoprene in the calibration solution
H_w = peak area of methoprene in the sample solution
I_r = peak area of the internal standard in the calibration solution
I_q = peak area of the internal standard in the sample solution
s = mass of methoprene in the calibration solution (mg)
w = mass of sample taken (mg)
P = purity of methoprene (g/kg)

(3) Calculation.

□

where:

$$R = \frac{Q}{c} \times 100\ \%$$

□

Q = content of free methoprene(g/kg)

c = content of total methoprene(g/kg), determined by method 414/CS/(M)/3

R = percentage of free methoprene relative to the total s-methoprene content (%)

□

Fig 1 Typical chromatogram of methoprene standard

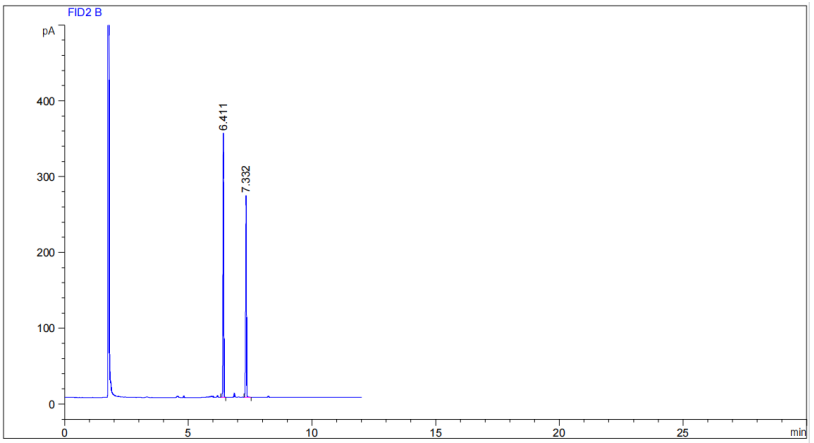
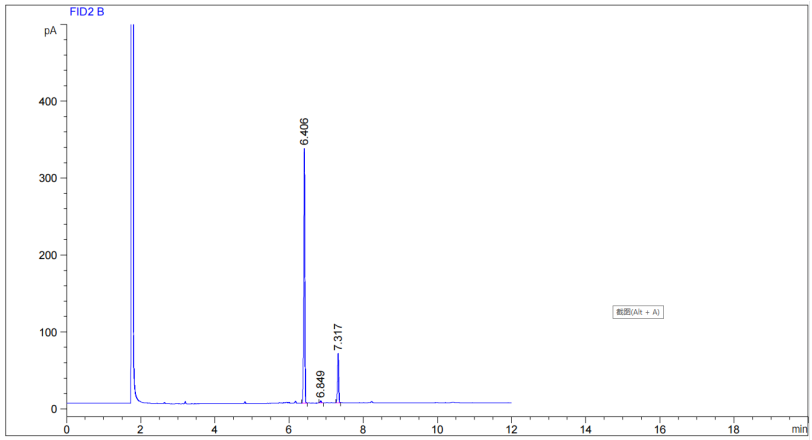


Fig 1 Typical chromatogram of methoprene in CS formulation


FID2 B
pA
400
300
200
100
0
6.406
6.849
7.317
0
2
4
6
8
10
12
14
16
18
min

3