### 5.2 Operation

The gas chromatograph shall be set up and operated according to the manufacturer's instructions.

## 6 Calibration solutions

Three calibration solutions with approximately the following methanol concentrations in FAME (3.3) shall be prepared as described below.

NOTE Three calibration solutions have proven sufficient in daily practise for a reliable coverage of the concentration range given in the scope of this method. For other concentration ranges, it is also possible to use other or more calibration solutions.

**6.1 Calibration solution A** (0,5 % (*m/m*) methanol in FAME).

Fill a 25 ml volumetric flask with 25 ml of FAME (3.3) and add (112 ± 0,1) mg (142 µl) methanol into the liquid phase using a syringe (4.3), the addition being made into the liquid phase. The exact masses shall be determined by weight measurement. It is necessary to ensure thorough mixing by vigorous shaking.

**6.2 Calibration solution B** (0,1 % (*m/m*) methanol in FAME).

Transfer 5 ml of calibration solution A into a 25 ml analytical flask and carefully fill to the mark with FAME (3.3).

**6.3 Calibration solution C** (0,01 % (*m/m*) methanol in FAME).

Transfer 1 ml of calibration solution B into a 10 ml analytical flask and fill to the mark with FAME (3.3).

## 7 Procedure

Two alternative procedures, the first using internal calibration and the second using external calibration are described in clauses 7.1 and 7.2, respectively.

### 7.1 Procedure A - Using internal calibration

This procedure is generally preferred when only a small number of samples is analyzed and when automatic head space equipment is not available. For manual procedure, see note in clause (4.10).

#### 7.1.1 Internal calibration

(5 ± 0,01) g from each calibration solution is transferred into a head space vial (4.1) and 5 µl of 2-propanol (3.2) is added into the liquid phase using a syringe (4.3), the addition being made into the liquid phase. The vials are then immediately crimped and shaken vigorously to ensure mixing.

Every 10 min, introduce into thermostatically-controlled bath or oven (4.12) a vial of the calibration solution which shall be kept there for exactly 45 min.

Preheat the gas syringe (4.4) to 60 °C in an oven (4.13). Sample 500 µl of gaseous phase (headspace) above the solution to be analysed and carry out the chromatographic analysis.

The calibration factor $F$ is calculated for each calibration solution according to equation (1), shall be expressed rounded to the nearest 0,01.

$$F = \frac{(C_n \times S_i)}{(C_i \times S_n)} \qquad (1)$$

where

$C_i$ is the 2-propanol content in the calibration solution, expressed in % (*m/m*);

(If 5 µl of 2-propanol are introduced into 5,0 g calibration solution, then $C_i$ = 0,0785 % (*m/m*)).

$C_m$ is the methanol content in the calibration solution, expressed in %(*m/m*);

$S_i$ is the peak area of 2-propanol;

$S_m$ is the peak area of methanol.

The calibration factor values obtained for the three reference solutions shall exhibit a coefficient of variation below 15 %. If this value is exceeded, the experimental set-up shall be inspected for errors and the calibration procedure shall be repeated starting from clause (6). The mean of these calibration factor values (in the region of 0,7) is used for the calculation described in clause 7.1.2.

### 7.1.2 Analysis and Calculation using internal calibration

The samples shall be prepared and analysed with exactly the same experimental conditions used in the calibration runs (7.1.1). The methanol content $C_m$ of a sample, expressed in % (*m/m*), is calculated according to equation (2) and rounded to two decimal places:

$$C_m = \frac{F \times S_m \times C_i}{S_i} \quad (2)$$

where

$F$ is the calibration factor obtained according to clause 7.1.1;

$S_m$ is the peak area of methanol;

$C_i$ is 2-propanol content added to the sample, expressed in % (*m/m*);

(If 5 µl of 2-propanol are introduced into 5,0 g sample, then $C_i$ = 0,0785 %(*m/m*)).

$S_i$ is the peak area of 2-propanol.

## 7.2 Procedure B - Using external calibration

This procedure is usually proferred when automatic head space equipment is used and a large number of sampled is analysed. It is not recommended to use external calibration when the analysis is executed manually, i.e. without automatic head space equipment.

### 7.2.1 External calibration

2 ml from each calibration solution is transferred into a head space vial. The vial shall be crimped immediately. The vials are then placed into the head space sampler and the analysis is started according to the manufacturer's instruction manual.

The calibration function is calculated using linear regression, using the methanol contents as dependent variable and the peak areas as independent variable. Using the resulting slope, $d$, and y-intercept, $e$, the regression function is then converted using equation (3):

$$C_m = a + b \times S_m \quad (3)$$

where

$C_m$ is the methanol content in % (*m/m*);

$a$ is the coefficient obtained from ( - $e/d$ );

$b$ is the coefficient obtained from ( $1/d$ );

$S_m$ is the area of the methanol peak;

$d$ is the slope of regression line;

$e$ is y intercept of regression line.

NOTE If the correlation coefficient is less than 0,95, the procedure should be inspected for errors and the calibration procedure should be repeated starting with clause 6.

#### 7.2.2 Analysis and calculation using external calibration

The samples shall be prepared and analysed with exactly the same experimental conditions used in the calibration runs (7.2.1). The methanol content $C_m$ of a sample, expressed in % ($m/m$), is calculated according to equation (3) and rounded to the nearest 0,01 % ($m/m$).

## 8 Precision

The precision data for procedure A and procedure B have both been obtained from a European interlaboratory trial organised in 1999. In this round robin exercise, no significant differences were observed between procedure A and procedure B (with automatic headspace system or with manual procedure) for the methanol concentration range of 0,01 % to 0,20 % ($m/m$).

### 8.1 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short time interval, shall not be greater than:

$$r = 0{,}056 \cdot X + 0{,}001 \qquad (4)$$

more than once out of 20 determinations ($X$ being the mean of the two results in question).

### 8.2 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories by different operators using different equipment, shall not be greater than:

$$R = 0{,}221 \cdot X + 0{,}003 \qquad (5)$$

more than once out of 20 determinations ($X$ being the mean of the two results in question).

## 9 Test report

The test report shall specify:

— all information necessary for the complete identification of the sample;

— the sampling method used if known;

— the test method used, with reference to this European standard;

— all operating details not specified in this European Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

— the test result(s) obtained, or if the repeatability has been checked, the final quoted result obtained.