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MAFF VALIDATED METHODS FOR THE ANALYSIS OF FOODSTUFFS

No. V18

VINYL CHLORIDE IN FOODS

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1. Scope and Field of Application

The method allows the determination of the vinyl chloride content of foodstuffs.

2. Definition

Vinyl chloride content: the content of vinyl chloride as determined by the method specified.

3. Principle

The vinyl chloride content of foodstuffs is determined by means of gas chromatography, using the "headspace" method.

4. Reagents

All reagents should be of recognised analytical grade unless specified otherwise.

4.1 Vinyl chloride, VC, of purity greater than 99.5%.

4.2 *N,N*-Dimethylacetamide, DMA, not containing any impurity with the same chromatographic retention time as VC or as the internal standard (**4.3**), under the conditions of the test.

4.3 Diethyl ether or *cis*-2-butene, in DMA (**4.2**) as the internal standard solution, ISS. These internal standards should not contain any impurity with the same chromatographic retention time as VC, under the conditions of the test.

5. Apparatus

5.1 Gas chromatograph, fitted with automatic headspace sampler or with facilities for manual sample injection.

5.2 Detector, flame ionisation or other suitable detector.

5.3 Gas chromatographic column, (see Appendix 1), meeting the following requirements:

5.3.1 It must be capable of separating the air peak, the VC peak of the standard solution (**6.1**) and the internal standard peak, if this is used;

5.3.2 The signal obtained with a solution containing 0.005 mg VC/litre or 0.005 mg VC/kg (**6.1**) must be equal to at least five times the background noise.

5.4 Sample phials or flasks, fitted with a silicone or butyl rubber septum. When using manual sampling techniques, the taking of a sample in the headspace with a syringe may cause a partial vacuum to form inside the phial or flask. Hence, for manual techniques where the phials are not pressurised before the sample is taken, the use of large phials is recommended.

5.5 Micro-syringes

5.6 Gas-tight syringe, for manual headspace sampling.

6. Procedure

Vinyl chloride is a hazardous substance and a gas at ambient temperature, therefore the preparation of solutions should be carried out in a well ventilated fume cupboard.

Take all the necessary precautions to ensure that no VC or DMA is lost.

It is highly recommended that when employing manual sampling techniques, an internal standard (**4.3**) should be used; when using an internal standard, the same solution should be utilised throughout the procedure.

6.1 Preparation of standard VC solutions

6.1.1 Concentrated standard VC solution, approximately 2000 mg/kg.

Weigh to an accuracy of 0.1 mg a suitable glass vessel and place in it a quantity (eg 50 ml) of DMA (**4.2**). Re- weigh. Add to the DMA a quantity (eg 0.1 g) of VC (**4.1**) in liquid or gas form, injecting it slowly into the DMA. The VC may also be added by bubbling it into the DMA, provided that a device is used which will prevent loss of DMA. Re-weigh to an accuracy of 0.1 mg. Wait 2 hr. to allow equilibrium to be attained. Keep the standard solution in a refrigerator.

6.1.2 Dilute standard VC solution A

Take a weighed amount of concentrated standard solution (**6.1.1**) and dilute to a known volume or a known weight, with ISS (**4.3**) or with DMA (**4.2**). The concentration of resultant diluted solution (Solution A) is expressed as mg/l or mg/kg respectively.

6.1.3 Dilute standard VC solution B

Repeat the procedure described above (**6.1.1 and 6.1.2**) to obtain a second diluted standard solution B with a concentration approximately equal to 0.02 mg of VC/l of ISS or DMA. Dispense this solution into

two phials (**5.4**). Seal the phials and proceed as described under 6.4 below.

6.2 Validation of standard VC solutions A and B

6.2.1 Calibration curve for Solution A

Prepare two series of seven phials (**5.4**): add to each phial, volumes of dilute standard VC solution A (**6.1.2**) and DMA (**4.2**) or ISS (**4.3**) such that the final concentrations of the duplicate solutions will be approximately equal to 0; 0.005; 0.010; 0.020; 0.030; 0.040; 0.050 mg/l of DMA. Seal the phials and proceed as described under 6.4 below. Accept the calibration curve thus obtained if it meets the criteria listed under 6.2.2 below.

6.2.2 Acceptability of calibration curve

(i) The repeatability of the responses as given in recommendation ISO R 5725 should be better than 0.002 mg of VC/l or kg of DMA;

(ii) The curve must be constructed from at least seven pairs of points. The curve should be calculated from these points by least square techniques.

(iii) The curve must be linear; that is, the standard deviation of the responses around the regression line divided by the mean value of all responses must not exceed 0.07.

6.2.3 Validation of Solution A

If the average of two gas chromatographic determinations relating to Solution B (**6.1.3**) do not differ by more than 5% from the corresponding point on the calibration curve for Solution A (**6.2.1, 6.2.2**), then Solution A is accepted. If the difference is greater than 5%, reject all the solutions obtained under 6.1 and 6.2, and repeat the procedure from the beginning.

6.3 Construction of the "addition" curve for samples

6.3.1 Homogeneous foodstuffs

Prepare two series of seven phials (**5.4**): add to each phial a quantity of sample, obtained from the foodstuff under investigation, of not less than 5 g. Try to ensure that an equal quantity is added to each phial. Close the phial immediately. Add to each phial such volumes of diluted standard VC solution (containing the internal standard if considered useful) as will give concentrations of added VC in the phials equal to 0; 0.005; 0.010; 0.020; 0.030; 0.040 and 0.050 mg/kg of foodstuff. Use diluted standard VC solutions (**6.1.2**) such that the ratio between the volume (µl) of this VC solution and the quantity (g) of foodstuff contained in the phial is as low as possible and not more than 5. Seal the phials and proceed as described under 6.4 below. Accept the "addition" curve thus obtained if it meets the criteria under 6.2.2(ii) and 6.2.2(iii) above.

6.3.2 Other foodstuffs

Prepare two series of seven phials (**5.4**): add to each phial a quantity of sample, obtained from the foodstuff under investigation, of not less than 5 g. Try to ensure that an equal quantity is added to each phial. Close the phial immediately. Add to each phial for each 5 g of sample 5 ml of an appropriate solvent (preferably distilled or demineralised water) containing internal standard (**4.3**) if considered useful, and such volumes of diluted standard VC solution as will give concentrations of added VC in the phials equal to 0; 0.005; 0.010; 0.020; 0.030; 0.040 and 0.050 mg/kg of foodstuff. Use diluted standard VC solution (**6.1.2**) such that the ratio between the volume (µl) of this VC solution and the quantity (g) of foodstuff contained in the phial is as low as possible and not more than 5. Seal the phials and proceed as described under 6.4 below. Accept the "addition" curve thus obtained if it meets the criteria under 6.2.2(ii) and 6.2.2(iii) above.

6.4 Gas chromatographic determinations

6.4.1 Put all the sealed phials in a waterbath for 2 hr at 60 ± 1°C to allow equilibrium to be attained. Agitate the phials avoiding contact between the contained liquid and the septum (**5.4**) to obtain a solution or a suspension as homogeneous as possible.

6.4.2 Take a sample from the headspace in the phial. When utilising manual sampling techniques, care should be exercised in obtaining a reproducible sample (**5.4**); in particular, the syringe should be prewarmed to the temperature of the sample. Measure the area (or the height) of the peaks corresponding to the VC (and to the internal standard, if used). Construct a graph in which the ordinate value shows the areas (or heights) of the VC peaks or the ratio of the areas (or heights) of VC peaks to the areas (or heights) of the internal standard peaks; and the abscissa value shows the quantities of VC added (mg) relative to the quantities of foodstuff weighed into each phial (kg). Projecting to zero peak area (or height), the intersect on the abscissa axis then shows the unknown concentration of VC in the sample under investigation (**Fig. 1**).

6.4.3 If necessary, remove from the column the excess DMA (4), using appropriate methods as soon as peaks from the DMA appear on the chromatogram.

6.5 Confirmation of the VC content

If the quantity of VC determined exceeds the limit in Annex II, paragraph 2 of the Council Directive 78/142/EEC, the results should be confirmed in one of the three ways 6.5.1-3 outlined below.

Fig 1

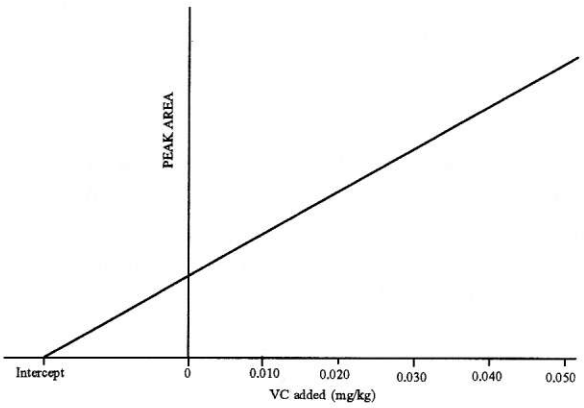

Fig. 1 A graph in which the ordinate value shows the areas of the VC peaks (or the ratio of the areas of VC peaks to the areas of the internal standard peaks); the abscissa value shows the quantities of VC added, related to the quantities of the sample of foodstuff weighed in each phial.

6.5.1 Change of stationary phase

Use at least one other column having a stationary phase of different polarity; this procedure should continue until a chromatogram is obtained with no evidence of overlap of the peaks corresponding to VC and/or internal standard with those corresponding to constituents of the foodstuff.

6.5.2 Change of detector

Use other detectors, e.g. a micro-electrolytic conductivity detector.

6.5.3 Use of mass spectrometry

The finding, that molecular ions with parent masses (m/e) of 62 and 64 are present in the ratio of 3:1, can be regarded with high probability as confirming that VC is present. In case of doubt, the total mass spectrum should be checked.

7. COSHH

Analysts are reminded that appropriate hazard and risk assessments required by the Control of Substances Hazardous to Health Regulations, 1988 (See "Control of Substances Hazardous to Health - Approved Code of Practice, Control of Substances Hazardous to Health Regulations, 1988") must be made before using this method.

8. Expression of Results

From the graph (**6.4.2**), read the observed level of VC and express as mg of VC per kg of foodstuff.

9. References

9.1 D O Biltcliffe and R Wood, The Determination of Vinyl Chloride in Foods : Collaborative Study, J. Assoc. Publ. Analysts, 1982, **20**,55- 65.

9.2 Ministry of Agriculture, Fisheries and Food, Food Safety Directorate, MAFF Validated Methods for the Analysis of Food, Introduction, General Considerations and Analytical Quality Control, J. Assoc. Publ. Analysts, 1992, **28**, 11-16

APPENDIX 1

GLC Columns and Conditions

1 Recommended Conditions

The following column and conditions were recommended by MAFF to the participants in the collaborative trial (Appendix 2):

3.0 m x 2 mm i.d. stainless steel column with 25% di- iso-decylphthalate and 0.5% Atpet 80 on 60- 70 mesh Diatomite C- AW HMDS;

carrier gas flow rate, 20 ml/min.;

column temperature 85°C (isothermal).

2 Suitable Condition

The following columns and conditions of gas- liquid chromatography were used by the eight laboratories participating in the collaborative trial[1] (Appendix 2).

Lab.1 3.0 m × 2 mm i.d. stainless steel column with 25% di-iso-decylphthalate and 0.5% Atpet 80 on 60-70 mesh Diatomite C-AW HMDS; nitrogen carrier gas, flow rate 20 ml/min.; column temperature 85°C; automatic injection.

Lab.2 As lab.1, except: manual injection.

Lab.3 As lab.1, except: 60-85 mesh Chromosorb W AW-DMCS; column temperature 50°C.

Lab.4 As lab.1, except: glass column; 60- 80 mesh Diatomite C-AW HMDS; nitrogen carrier gas, flowrate 30 ml/min.; manual injection.

Lab.5 As lab.1, except: 10 ft × 1/4 in i.d. column; 60-85 mesh Chromosorb W AW DMCS; nitrogen carrier gas, flow rate 75 ml/min.; column temperature 45°C; manual injection.

Lab.6 5 ft × 4.5 mm i.d. glass column with 0.2% carbowax 1500 on Carbopak C; nitrogen carrier gas, flow rate 20 ml/min.; column temperature 70°C; manual injection.

Lab.7 As lab.1, except: nitrogen carrier gas, flow rate 22 ml/min.; column temperature 65°C; manual injection.

Lab.8 As lab.1, except: nitrogen carrier gas, flow rate 24 ml/min.; column temperature 83°C; manual injection.

APPENDIX 2

Analytical Quality Control

General principles of analytical quality control are outlined in protocol V.0 of the series[2].

A1 Repeatability

The absolute difference between two test results obtained under repeatability conditions should not be greater than the repeatability, r, deduced from the collaborative trial data summarised below (Table 1). The observed repeatability, r, always fell below the value of 3 μg/kg given in the adopted EC Directive on the method of analysis of vinyl chloride in foodstuffs. Overall, r may be taken to be 3 μg/kg. This corresponds to a standard deviation of repeatability, S_r, of approximately 1 μg/kg. At this precision, the relative standard deviation of repeatability (coefficient of variance of repeatability), RSD_r, is about 10% at levels of 10 μg/kg.

A2 Reproducibility

The absolute difference between two test results carried out under reproducibility conditions should not be greater than the reproducibility, R, deduced from the collaborative trial data summarised below (Table 1). R may be taken as 7 μg/kg. This precision corresponds to a relative standard deviation of reproducibility (coefficient of variance of reproducibility) of about 25% at levels of 10 μg/kg.

A3 Trueness (Bias)

The samples consisted of orange drink spiked with known levels of VC; the recoveries were lower than might have been expected, being of the order of 50%. Poor recovery has been consistently observed from an orange drink matrix, in contrast to oil samples where the measured levels are usually very close to the level of spiking.

A4 Limit of Detection

This limit has not been established, but the collaborative trial data suggests an accuracy which, if maintained, corresponds to a lower limit of roughly 1 μg/kg for a single determination.

A5 Statistical Data Derived from the Results of Interlaboratory Tests

Three samples of orange drink were prepared by spiking with VC at levels of 5, 15 and 30 µg/kg. Participants in the collaborative trial each analysed the three samples twice.

Table 1 summarises the statistical data; the levels of VC are expressed in µg/kg.

TABLE 1

Statistical Analysis of Vinyl Chloride (µg/kg) in Orange Drink Samples

Orange Drink sample	A	B	C
Number of Laboratories retained after eliminating outliers	6	6	6
Number of Laboratories eliminated as outliers	2	2	2
Number of results accepted after eliminating outliers	6	6	6
LEVEL OF ANALYTE			
Level of added analyte	5	15	30
Mean observed value $\bar{x}$	4.05	6.16	16.09
REPEATABILITY			
Standard Deviation S_r	0.34	0.58	0.93
Relative Standard Deviation RSD_r(%)	8.5	9.3	5.8
Repeatability r [2.8 x S_r]	0.96	1.61	2.6
REPRODUCIBILITY			
Standard Deviation S_R	1.0	2.1	2.5
Relative Standard Deviation RSD_R(%)	25	33	16
Reproducibility R [2.8 x S_R]	2.8	5.8	7.0

A6 Key to Table 1

Symbol	Definition
$\bar{x}$	Overall mean value
S_r	The standard deviation of repeatability
RSD_r	The relative standard deviation of repeatability, expressed as a percentage of the mean (coefficient of variance of repeatability CV_r)
r	Repeatability
S_R	The standard deviation of reproducibility
RSD_R	The relative standard deviation of reproducibility, expressed as a percentage of the mean (coefficient of variance of reproducibility CV_R)
R	Reproducibility