
**Vulcanized rubber — Determination
of free sulfur by gas chromatography
(GC) and high performance liquid
chromatography (HPLC)**

*Caoutchoucs vulcanisés — Dosage du soufre libre par
chromatographie en phase gazeuse (CG) et chromatographie en
phase liquide haute performance (HPLC)*

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Contents

	Page
Foreword	iv
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 Principle	1
5 Apparatus	2
6 Reagents and materials	3
6.1 Standard solutions	3
6.2 Materials for preparation of samples	3
6.3 Reagents used in GC	3
6.4 Elution solvents used in HPLC	4
7 Preparation of standard solutions and calibration curves	4
7.1 Standard addition method	4
7.2 External calibration method	4
8 Preparation of samples	5
9 Chromatographic procedure	5
9.1 Method A by GC procedure	5
9.2 Method B by HPLC procedure	5
9.2.1 General	5
9.2.2 Standard addition method	6
9.2.3 External calibration method	6
10 Sample analysis and calculation	6
10.1 General	6
10.2 Calculation using standard addition method	7
10.3 Sample analysis and calculation using external calibration method	7
11 Precision	8
12 Test report	8
Annex A (normative) Calibration using standard addition method	9
Annex B (normative) Calibration using external standard solutions	10
Annex C (informative) Examples of chromatogram	11
Annex D (informative) Precision	12
Bibliography	17

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 45, *Rubber and rubber products*, Subcommittee SC 2, *Testing and analysis*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Vulcanized rubber — Determination of free sulfur by gas chromatography (GC) and high performance liquid chromatography (HPLC)

WARNING 1 — Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to determine the applicability of any other restrictions.

WARNING 2 — Certain procedures specified in this document might involve the use or generation of substances, or the generation of waste, that could constitute a local environmental hazard. Reference should be made to appropriate documentation on safe handling and disposal after use.

1 Scope

This document specifies chromatographic methods for the determination of the inorganic free sulfur (S_x) not consumed during the vulcanization of the rubber. This method is used primarily to compare the free sulfur content in different samples.

NOTE 1 In this document, “free sulfur” is used instead of “inorganic free sulfur”.

NOTE 2 The residual curing agent containing sulfur is not concerned by this measure.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 1407:2011, *Rubber — Determination of solvent extract*

3 Terms and definitions

There are no term and definition in this document.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <http://www.electropedia.org/>

4 Principle

The free sulfur is quantified following two techniques: gas chromatography/mass spectrometric detector (GC-MS) or gas chromatography/flame ionization detector (GC/FID) (method A) and high performance liquid chromatography/ultraviolet detector (HPLC/UV) (method B).

A sample of vulcanized rubber is first extracted using an appropriate solvent according to ISO 1407:2011 (Method B). A known quantity of triphenylphosphine (TPP) is added to the obtained solution. TPP reacts with free sulfur to form a stable compound, triphenylphosphine sulfide (TPPS). See [Figure 1](#).

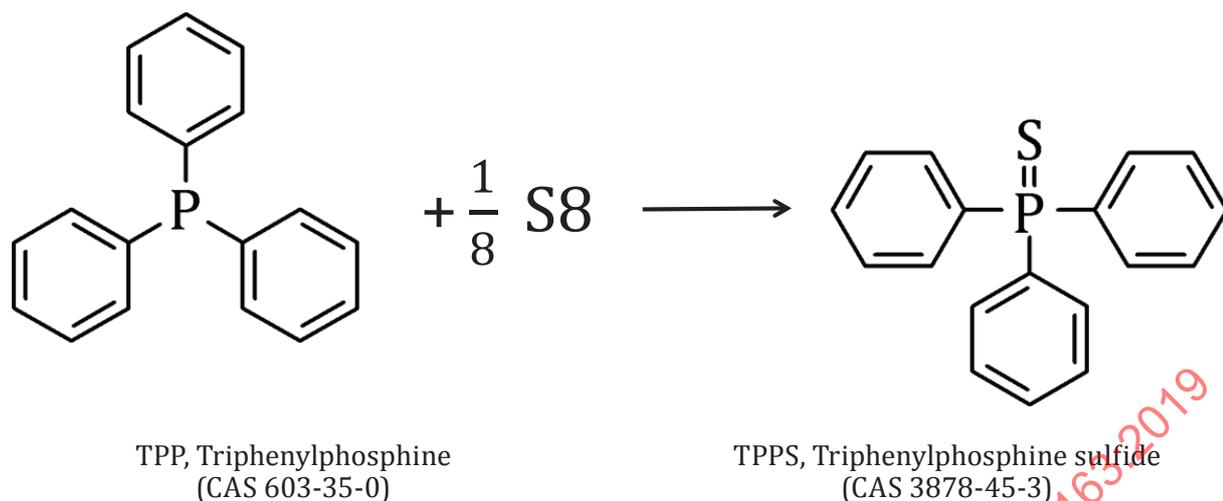


Figure 1 — TPP reaction with free sulfur for derivatization

TPPS, which contains aromatic rings, offers the following advantages:

- firstly, to have a more active chromophore in UV than sulfur, therefore being more easily detectable in HPLC/UV;
- secondly, to have sufficient thermal stability to be injected in GC.

The following calibration methods are possible for both types of chromatography (GC and HPLC). The preparation of samples is the same for GC and HPLC.

- Standard addition method is the preferred calibration method, used to correct matrix effects and when samples contain unknown contents of free sulfur.
- External calibration is used in case of absence of matrix effect and to check known contents of free sulfur.

5 Apparatus

5.1 Balance, precision 0,01 mg.

5.2 Soxhlet extractor.

5.3 Material for gas chromatography (Method A).

5.3.1 Injector for either split or splitless injection.

5.3.2 Mass spectrometric (MS) detector or flame ionization detector (FID).

5.3.3 Mass spectrometer with the following specifications:

- transfer line temperature: 300 °C;
- ionizing energy: 70 eV;
- scan range: 33 m/z to 600 m/z;
- analysis mode: full scan.

5.3.4 Data system, using an acquisition and a data processing software.

5.3.5 Apolar capillary column.

A column 5 % diphenyl-dimethylpolysiloxane of 30 m length, 0,25 mm diameter and 0,25 µm thickness film, is usually appropriate.

The use of an autosampler is recommended for a better precision.

5.4 Material for liquid chromatography (Method B).

5.4.1 Solvent pump, allowing the use of gradient.

5.4.2 Injector, allowing injection of variable volumes.

5.4.3 Ultraviolet (UV) detector.

5.4.4 Data system, using an acquisition and a data processing software.

5.4.5 Column, for example, a cartridge with C18 type silica-bound.

A column of 15 cm length, 4,6 mm diameter and 5 µm particle size, is usually appropriate.

6 Reagents and materials

6.1 Standard solutions

6.1.1 TPP, analytical grade.

6.1.2 TPPS, analytical grade.

6.1.3 Tetrahydrofuran (THF), HPLC grade.

6.2 Materials for preparation of samples

6.2.1 Single use polytetrafluoroethylene (PTFE) filters, 25 mm diameter, 0,45 µm porosity.

6.2.2 Solvent for extraction, according to ISO 1407:2011, Table A.1, or any suitable solvent (e.g. dichloromethane).

6.2.3 THF, HPLC grade.

6.3 Reagents used in GC

6.3.1 Helium, purity $\geq 99,999$ % or purity $\geq 99,999$ 9 % quality, as carrier gas (hydrogen can also be used).

6.3.2 Air and hydrogen, purity $\geq 99,99$ %.

6.3.3 Syringe rinsing solvent: cyclohexane of analytical grade (or other solvent for the sample dissolution).

6.3.4 **Rinsing solvent used between each injection series:** THF, HPLC grade.

6.4 Elution solvents used in HPLC

6.4.1 **Water**, ultrapure quality (resistivity $\geq 18 \text{ M}\Omega\cdot\text{cm}$) used for the preparation of ammonium acetate buffer solution (pH 6,7).

6.4.2 **Acetonitrile**, HPLC grade.

6.4.3 **Ammonium acetate**, analytical grade, used for the preparation of buffer solution, using 10^{-2} mol/l of ammonium acetate.

7 Preparation of standard solutions and calibration curves

7.1 Standard addition method

The standard solutions of standard addition method shall be prepared as specified below.

Add standard solutions varying in concentration to the sample solution into several even aliquots in separate volumetric flasks of the same volume.

The standard solutions A, B, C, D and E correspond to the concentration range from 5×10^{-4} mol/l to 5×10^{-3} mol/l of TPPS. The preparation of the standard solutions shall be carried out in accordance with [Table A.1](#).

The concentration range shall be adapted in function of the requested quantification limit.

Add 0,5 ml of each standard solution to the sample solution (F, prepared according to [Clause 8](#)):

- G = F (0,5 ml) + A (0,5 ml);
- H = F (0,5 ml) + B (0,5 ml);
- I = F (0,5 ml) + C (0,5 ml);
- J = F (0,5 ml) + D (0,5 ml);
- K = F (0,5 ml) + E (0,5 ml).

The injected volume is 1 μl in GC (Method A) and 20 μl in HPLC (Method B).

The calibration curve is obtained by plotting the peak area of G to K solutions versus the concentrations of the standards TPPS, A to E, see example in [Figure 2](#).

In the absence of autosampler, repeat the injections three times and report the average of the three values.

7.2 External calibration method

The various solutions are prepared and correspond to the concentration range from 5×10^{-3} mol/l to 6×10^{-5} mol/l of TPPS. The preparation of the standard solutions shall be carried out in accordance with [Table B.1](#).

The concentration range shall be adapted in function of the requested quantification limit.

The injected volume is 1 μl in GC (Method A) and 20 μl in HPLC (Method B).

The calibration curves are obtained by plotting the peak area function of the initial concentration of TPPS.

In the absence of autosampler, repeat the injections three times and report the average of the three values.

8 Preparation of samples

Weigh a test piece of vulcanized rubber (4 g is currently used), cut it into pieces and extract according to ISO 1407:2011, Method B.

Evaporate the obtained solution under partial vacuum using a rotary evaporator. Dilute the residue using 5 ml of THF.

NOTE The use of SPE (solid-phase extractor) cartridge, having a volume capacity of approximately 2 ml or 5 ml, can improve the purification of the extract.

Introduce the solution in a flask of 10 ml; add 4 ml of solution of TPP 10^{-1} mol/l to this solution. Complete up to 10 ml with THF. Filtrate with PTFE filter.

In order to have a total derivatization, it might be necessary to use a larger volume of TPP to be in excess related to free sulfur in the sample.

The tests shall be duplicated. Two test pieces per sample are extracted and derivatized.

9 Chromatographic procedure

9.1 Method A by GC procedure

The split ratio is 42 and the split flow is 50 ml/min.

The injector temperature is 250 °C.

The flowrate of the carrier gas (helium) is set at 1,2 ml/min.

In the case of a FID detector, the flowrate of air and hydrogen are 450 ml/min and 40 ml/min respectively. The detector temperature is 320 °C. The make-up could be adjusted.

The injected quantity is 1 µl.

The column oven temperature program is shown in [Table 1](#).

Table 1 — Column oven temperature program

Oven ramp	°C /min	Next °C	Hold min
Initial		50	0,00
Ramp 1	25,00	150	0,50
Ramp 2	10,00	250	0,00
Ramp 3	25,00	320	10,00
Post run	—	0,00	0,00

NOTE For information, the retention time for TPP and TPPS is 12,75 min and 16,10 min respectively.

An example of chromatogram for method A is found in [Figure C.1](#).

9.2 Method B by HPLC procedure

9.2.1 General

Two elution solvents are used in this method.

The temperature of the column is standard laboratory temperature.

The wavelength of detection is fixed to 260 nm.

The gradient elution program used is the following:

- solvent A: buffer aqueous solution at pH = 6,7, using ammonium acetate;
- solvent B: acetonitrile;
- flowrate: 1 ml/min.

NOTE 1 Solvents can be continuously degassed.

NOTE 2 For information, the retention time for TPPS and TPP is 8,96 min and 11,96 min respectively.

An example of chromatogram for method B is found in [Figure C.2](#).

9.2.2 Standard addition method

The operating conditions for standard solutions including the sample are given in [Table 2](#).

NOTE The gradient condition can be adapted depending on the sample analysed.

Table 2 — Operating conditions for sample solutions

Time <i>t</i> min	Solvent A %	Solvent B %
<i>t</i> = 0	50	50
<i>t</i> = 12 min	0	100
<i>t</i> = 30 min	0	100
<i>t</i> = 35 min	50	50
<i>t</i> = 45 min	50	50

9.2.3 External calibration method

The operating conditions for external calibration solutions are given in [Table 3](#).

Table 3 — Operating conditions for external calibration solutions

Time <i>t</i> min	Solvent A %	Solvent B %
<i>t</i> = 0	50	50
<i>t</i> = 12 min	0	100
<i>t</i> = 13 min	50	50
<i>t</i> = 20 min	50	50

The operating conditions for the sample solution are given in [Table 2](#).

10 Sample analysis and calculation

10.1 General

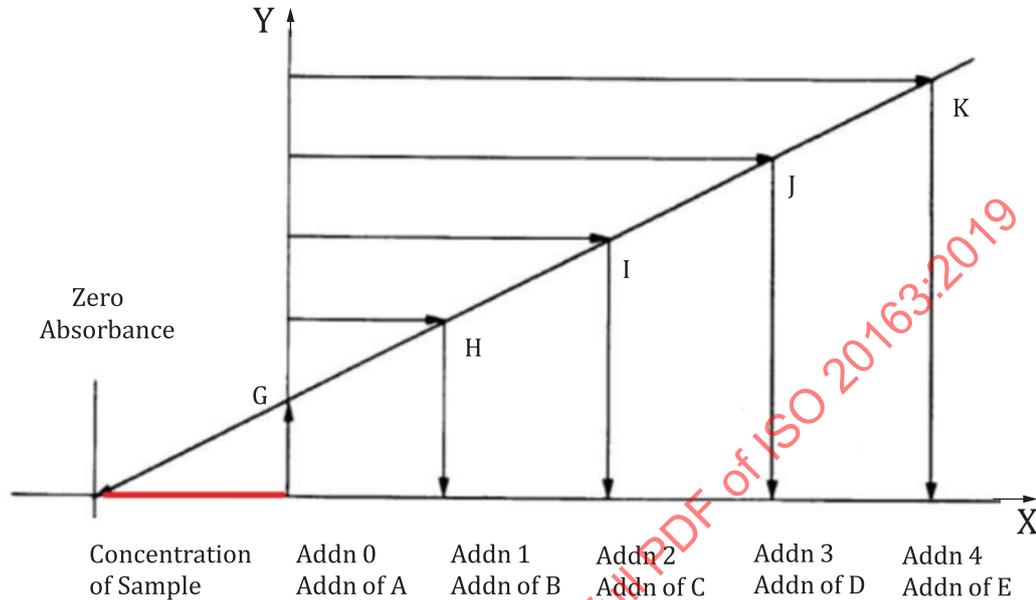
Check that the calibration curve is linear and the determination coefficient r^2 is greater than 0,99.

In order to obtain quantitative results, basic chromatographic rules shall be respected in term of resolution, coelution, etc.

Check in the obtained chromatogram that the derivatization reaction is complete, i.e. the TPP peak is saturated. Examples of chromatograms are given in [Figure C.1](#) (GC) and [Figure C.2](#) (HPLC).

10.2 Calculation using standard addition method

The concentration of TPPS shall be calculated, in mol/l, using the equation of the calibration curve typically $y = a x + b$, where linear regression is performed to the point on the abscissa x-axis at which $y = 0$. Using the slope (a) and y-intercept (b), this concentration x will be $x = \text{abscissa } (-b/a)$.



Key

X concentration
Y absorbance

Figure 2 — Standard addition calibration curve

The content of free sulfur in the test piece shall be calculated, in $\mu\text{g/g}$ (ppm), using [Formula \(1\)](#):

$$\text{Free sulfur content} = c_{\text{TPPS}} \times V \times M \times 10^6 / m \quad (1)$$

where

c_{TPPS} is the TPPS concentration in mol/l;

V is the volume of TPPS solution obtained after derivatization of the sample (10×10^{-3} l);

M is the atomic weight of sulfur (32,06 g/mol);

m is the mass of the test piece in g.

A test result shall be the average of two tests in either of method A or method B.

10.3 Sample analysis and calculation using external calibration method

Once the calibration system has been established, run the sample extract prepared in accordance with [Clause 8](#) using the procedure described in [9.1](#) (Method A) or [9.2](#) (Method B).

The concentration of TPPS shall be calculated, in mol/l, using the equation of the calibration curve and the TPPS area obtained for the test piece.

The content of free sulfur in the test piece shall be calculated, in $\mu\text{g/g}$ (ppm), using the [Formula \(1\)](#).

A test result shall be the average of two tests in either of method A or method B.

11 Precision

For precision, see [Annex D](#).

12 Test report

The test report shall include the following information:

- a) Sample details:
 - 1) full description of the sample;
 - 2) the extraction solvent used;
 - 3) extract rate (for information);
- b) Test method:
 - 1) a full reference to the test method used, i.e. ISO 20163:2019;
 - 2) the test procedure used, i.e. method A (GC-MS or GC/FID) or method B (HPLC/UV);
 - 3) the calibration procedure used, i.e. standard addition method or external calibration;
- c) Test details: details of any procedures not specified in this document;
- d) Test results:
 - 1) the number of test pieces used;
 - 2) the mean results, expressed in $\mu\text{g/g}$ (ppm), and rounded to one place of decimals;
- e) Date(s) of test.

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Annex A (normative)

Calibration using standard addition method

The solutions are prepared by successive dilutions of a TPPS solution of 10^{-2} mol/l, obtained by dissolving 294 mg of TPPS, exact to 0,01 mg (molecular weight $M = 294,36$ g/mol) in 100 ml of THF.

Take off 10 ml of the solution and introduce it into a flask of 100 ml, then complete with THF up to the mark. This dilution with THF gives a solution of 10^{-3} mol/l.

NOTE The order of injection standard solutions is generally from the less concentrated to the more concentrated.

Table A.1 — Steps for preparation of the standard solutions for the calibration range

References	Solutions	TPPS concentration mol/l
A	5 ml of 0 mol/l solution	0
B	5 ml of 10^{-3} mol/l solution then complete up to 10 ml	5×10^{-4}
C	1 ml of 10^{-2} mol/l solution then complete up to 10 ml	1×10^{-3}
D	3 ml of 10^{-2} mol/l solution then complete up to 10 ml	3×10^{-3}
E	5 ml of 10^{-2} mol/l solution then complete up to 10 ml	5×10^{-3}

The injected volume is 1 μ l in GC (method A) and 20 μ l in HPLC (method B).

Annex B (normative)

Calibration using external standard solutions

The solutions are prepared by successive dilutions of a TPPS solution of 10^{-2} mol/l, obtained by dissolving 294 mg of TPPS, exact to 0,01 mg (molecular weight $M = 294,36$ g/mol) in 100 ml of THF.

Take off 10 ml of the solution and introduce it into a flask of 100 ml, then complete with THF up to the mark. This dilution with THF gives a solution of 10^{-3} mol/l.

NOTE The order of injection standard solutions is generally from the less concentrated to the more concentrated.

Table B.1 — Steps for preparation of the standard solutions for the calibration range

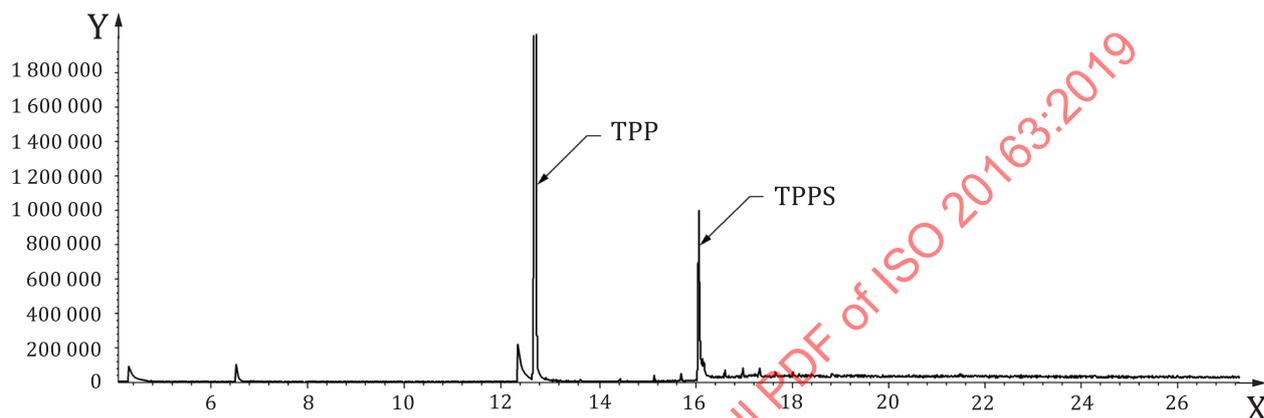
Solutions	TPPS concentration mol/l
5 ml of 10^{-2} mol/l solution then complete up to 10 ml	5×10^{-3}
2 ml of 10^{-2} mol/l solution then complete up to 10 ml	2×10^{-3}
1 ml of 10^{-2} mol/l solution then complete up to 10 ml	1×10^{-3}
5 ml of 10^{-3} mol/l solution then complete up to 10 ml	5×10^{-4}
3 ml of 10^{-3} mol/l solution then complete up to 10 ml	3×10^{-4}
6 ml of 10^{-3} mol/l solution then complete up to 100 ml	6×10^{-5}

The injected volume is 1 μ l in GC (Method A) and 20 μ l in HPLC (Method B).

Annex C (informative)

Examples of chromatogram

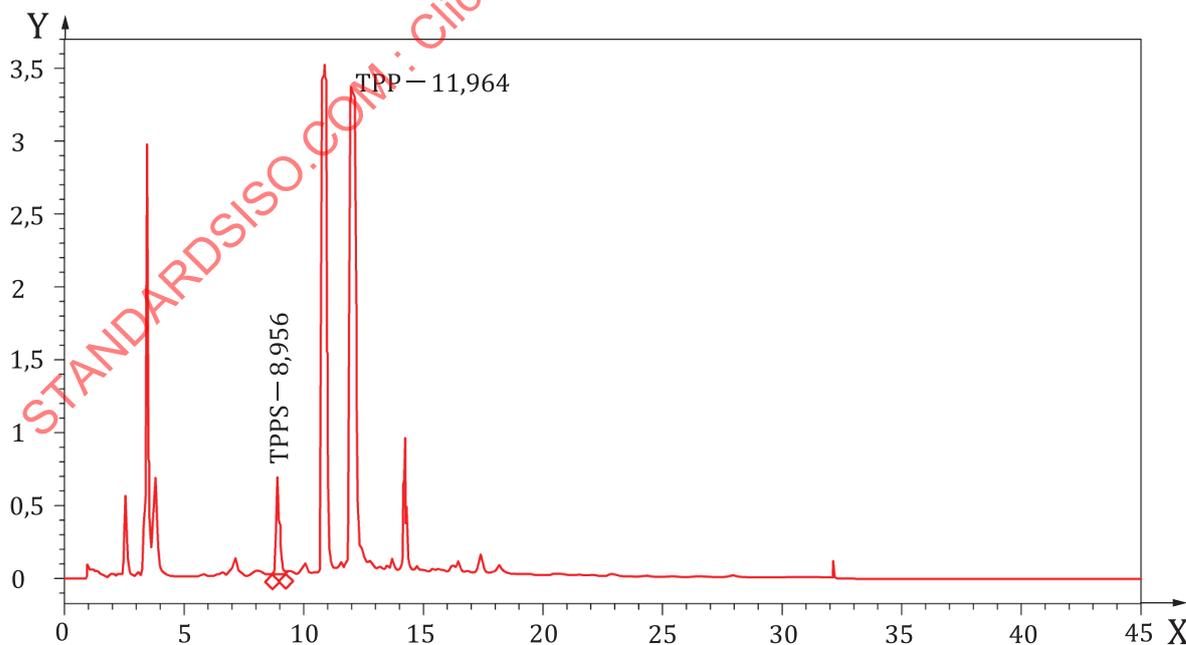
[Figure C.1](#) and [Figure C.2](#) are examples of chromatograms for method A and method B, respectively.



Key

X time (min)
Y abundance

Figure C.1 — Example of GC chromatogram (method A)



Key

X time (min)
Y abundance

Figure C.2 — Example of HPLC chromatogram (method B)

Annex D (informative)

Precision

D.1 General

Two interlaboratory test programs (ITPs) for precision evaluation for free sulfur content were carried out using respectively two calibration methods; the first in 2016 (external calibration method) and the second in 2017 (standard addition method), using the precision procedures and guidelines as described in ISO 19983:2017 Method A.

The ITP's were conducted using two compounds based on NR (conventional vulcanization) and EPDM (efficient vulcanization). They were tested using methods A (GC) and B (HPLC).

A TPPS standard of known concentration (32,8 mg/l), prepared by one laboratory and sealed for transportation, is also tested to control the analyses.

The testing was conducted on a day 1 vs day 2 repeatability basis, with 7 days between the day measurements. On each day, the test is duplicated and the test result is the mean value of two individual determinations of sulfur content by method A or B.

D.2 Interlaboratory test program (ITP) No 1 (2016)

D.2.1 Four laboratories participated in the 2016 ITP. A test result represents the mean for two individual determinations of free sulfur content obtained on each of material (NR and EPDM) on two different test days, seven days apart.

General statements for the use of the precision results for free sulfur in NR and EPDM are given in terms of both the absolute precision r_D and R , and the relative precision, (r_D) and (R) . The precision data resulting from the ITP, using the outlier replacement and outlier deletion procedures, are set forth in [Table D.1](#) for the method A (GC) and in [Table D.2](#) for the method B (HPLC).

The precision results as determined by this ITP may not be applied to acceptance or rejection testing for any group of materials or products without documentation that the results of this precision evaluation actually apply to the products or materials tested.

The 2016 ITP repeatability and reproducibility statements are as follows.

- *Repeatability*: The within-day repeatability has been established for each material by the values found in [Table D.3](#) or [D.4](#). Two single test results (obtained by the proper use of this standard) that differ by more than the tabulated values for r , in measurement units and (r) , in percent, shall be considered as suspect, i.e. to have come from different populations. Such a decision suggests that some appropriate investigative action be taken (see ISO 19983:2017, Annex E).
- *Day-to-day repeatability*: The day-to-day repeatability, or local domain precision, has been established for each material by the values found in [Table D.1](#) or [D.2](#). Two single test results (obtained by the proper use of this standard) that differ by more than the tabulated values for r_D , in measurement units and (r_D) , in percent, shall be considered as suspect, i.e. to have come from different populations. Such a decision suggests that some appropriate investigative action be taken (see ISO 19983:2017, Annex E).
- *Reproducibility*: The reproducibility, or global domain precision, has been established for each material by the values found in [Table D.1](#) or [D.2](#). Two single test results obtained in different

laboratories (by the proper use of this standard) that differ by more than the tabulated values for R , in measurement units and (R) , in percent, shall be considered as suspect, i.e. to have come from different populations. Such a decision suggests that some appropriate investigative action be taken (see ISO 19983:2017, Annex E).

Table D.1 — Precision for free sulfur using method A (GC) - ITP No. 1 (2016)

Material	Mean value (ppm)	Within laboratory, within-day			Within laboratory, day-to-day			Between laboratories			No of laboratories ^a
		s_r	r	(r)	s_{rD}	r_D	(r_D)	s_R	R	(R)	
NR	55,2	10,7	30,4	55,1	10,7	30,4	55,1	20,3	57,4	104,1	4
EPDM	112,3	19,9	56,3	50,2	36,5	103,4	92,1	60,0	169,8	151,2	4
TTPS (32,8 mg/l)	30,0 mg/l				3,8	10,6	35,5	4,3	12,1	40,4	4

^a Number of laboratories after deletion of outliers.
 s_r = within-day within laboratory standard deviation (in measurement units)
 r = within-day repeatability (in measurement units)
 (r) = within-day repeatability (in percent of mean level)
 s_{rD} = day-to-day within laboratory standard deviation (pooled over all laboratories, in measurement units)
 r_D = day-to-day repeatability (in measurement units)
 (r_D) = day-to-day repeatability (in percent of mean level)
 s_R = between-laboratory standard deviation (for total between laboratory variation, in measurement units)
 R = reproducibility (in measurement units)
 (R) = reproducibility (in percent of mean level)

Table D.2 — Precision for free sulfur using method B (HPLC) - ITP No. 1 (2016)

Material	Mean value (ppm)	Within laboratory, within-day			Within laboratory, day-to-day			Between laboratories			No of laboratories ^a
		s_r	r	(r)	s_{rD}	r_D	(r_D)	s_R	R	(R)	
NR	31,1	6,7	19,1	61,5	7,6	21,5	69,4	10,7	30,2	97,4	4
EPDM	99,9	15,4	43,5	43,5	25,0	70,6	70,7	35,8	101,2	101,2	4
TTPS (32,8 mg/l)	31,9 mg/l				0,4	1,2	3,8	0,7	2,0	6,3	4

^a Number of laboratories after deletion of outliers.
 s_r = within-day within laboratory standard deviation (in measurement units)
 r = within-day repeatability (in measurement units)
 (r) = within-day repeatability (in percent of mean level)
 s_{rD} = day-to-day within laboratory standard deviation (pooled over all laboratories, in measurement units)
 r_D = day-to-day repeatability (in measurement units)
 (r_D) = day-to-day repeatability (in percent of mean level)
 s_R = between-laboratory standard deviation (for total between laboratory variation, in measurement units)
 R = reproducibility (in measurement units)
 (R) = reproducibility (in percent of mean level)