# A Method for Evaluation of Volatile Sulfur-Containing Substances in Preparations for Intravenous Infusions

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Gas chromatographic method for measuring volatile sulfur compounds in solutions for intravenous infusions is proposed.

**Key Words:** *volatile sulfur compounds; gas chromatography*

The quality and safety of any preparation are largely determined by the content of impurities getting into the dosage form with active and accessory substances or forming during storage or as a result of oxidative, photochemical, and other processes. The composition of these admixtures is as a rule sufficiently well known, and the norm-setting analytical documents include the analytical methods for measurements of impurities in a drug.

On the other hand, impurities can accumulate because of incorrect choice of package materials (as a result of reaction between the package material and drug components or of chemical processes in the package proper). Evaluation of the content of these impurities can be carried out at the stage of drug development (this control is ruled out during commercial manufacture). The presence of impurities not characteristic of the active and accessory substances in some drugs intended for intravenous infusions (rheopolygluquin, Ringer-Locke solution, Trisol, 5% glucose solutions) and in preparations packed in glass bottles with rubber caps can be detected organoleptically (directly after opening of the bottle: by the odor characteristic of volatile sulfur-containing substances).

We developed an analytical method for detection of volatile sulfur compounds in solutions for intravenous infusions.

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Dimethylsulfide (DMSO; Merc; Cat. No. 200-846-2; basic substance content at least 99.0%) and diethylsulfide (DES; Aldrich; Cat. No. 238317-25G; basic substance content at least 96.0%) were used.

For preparation, selection, and introduction of an equilibrium vapor phase into the chromatograph, 10 ml preparation was placed into a 20-ml flask and 100 μl internal reference solution was added.

The internal reference solution was prepared as follows: 20 ml absolute ethanol was placed into a 25-ml flask, 250 μl DES was added with a microsyringe, the volume was brought to 25 ml with absolute ethanol and mixed. The resultant DES concentration was 8.42 mg/ml.

A total of 20 ml 40% ethanol was placed into a 25-ml flask, 100 μl resultant DES solution was added, the volume of the solution was brought to 25 ml with 40% ethanol, and mixed. The resultant DES concentration was 33.68 ng/μl.

Calibration solutions were then prepared. About 9 ml absolute ethanol was placed into a 10-ml flask, 30 μl DMSO was added with a cold microsyringe, the volume of the solution was brought to 10 ml with absolute ethanol and mixed. The resultant DMSO concentration was 2.51 mg/ml.

A total of 20 ml 40% ethanol was placed into a 25-ml flask, 2.5 ml resultant DMSO solution was added, the volume of the solution was brought to 25 ml with 40% ethanol and mixed. The resultant DMSO concentration was 251 ng/μl.

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**TABLE 1.** Calibration Samples

| Vial No. | Volume of 0.9% NaCl solution, ml | Volume of DMSO solution, μl | Volume of DES solution, μl | DMSO concentration in vial, μg/ml | DES concentration in vial, μg/ml |
|---|---|---|---|---|---|
| 1 | 10.0 | 0 | 100 | 0 | 0.3368 |
| 2 | 10.0 | 10 | 100 | 0.251 | 0.3368 |
| 3 | 10.0 | 20 | 100 | 0.502 | 0.3368 |
| 4 | 10.0 | 30 | 100 | 0.753 | 0.3368 |
| 5 | 10.0 | 40 | 100 | 1.004 | 0.3368 |
| 6 | 10.0 | 50 | 100 | 1.255 | 0.3368 |
| 7 | 10.0 | 60 | 100 | 1.506 | 0.3368 |
| 8 | 10.0 | 70 | 100 | 1.757 | 0.3368 |
| 9 | 10.0 | 80 | 100 | 2.008 | 0.3368 |
| 10 | 10.0 | 90 | 100 | 2.259 | 0.3368 |

Calibration reference specimens were prepared as follows. 10 ml 0.9% NaCl was placed into each of 10 20-ml flasks, calibration solution and internal reference solution (Table 1) were added, and sealed immediately.

Equilibrium vapor phase was prepared on a GC-14B gas chromatograph equipped with an FPD-14 flame photometric detector, HSS-2B attachment for preparation, selection, and introduction of equilibrium vapor phase into the chromatograph, and a C-R7a integrator (Shimadzu). The test and calibration samples were analyzed by chromatography.

Equilibrium vapor phase was prepared at the temperature of incubation (55°C, 30-min incubation), the injector syringe temperature being 70°C, the volume of injected sample 0.8 ml.

A DB-5 capillary quartz column 50 m×0.53 mm was used (5% phenylmethylpolysiloxane; immobile phase thickness 5 μ); the conditions of the analysis were as follows: column temperature 50°C, sample introduction block $t$=120°C, detector block $t$=180°C, thermostat $t$=200°C, carrier gas (He) flow rate 8 ml/min, flow division 1:20; maximum optic filter transmission 393.2 nm.

The results of chromatographic analysis of DMSO calibration samples are presented in Table 2.

Calibration curve presenting the relationship between the ratio of DMSO to internal standard (DES) peak areas and the DMSO concentration was plotted.

In order to evaluate the concentration of sulfur-containing substances in conversion to DMSO, 50 μl analyzed preparation was placed into the vial for preparation, selection, and introduction of equilibrium vapor phase into the chromatograph, 10 ml 0.9% NaCl solution and 100 μl DES solution were added, mixed, and analyzed by chromatography. The

**TABLE 2.** Results of Chromatography of DMSO Calibration Samples

| Sample | DMSO peak area, cm² | DES peak area, cm² | $S_{DMSO}/S_{DES}$ | DMSO concentration in sample |
|---|---|---|---|---|
| 1 | 0 | 91 844 | 0 | 0 |
| 2 | 3318 | 108 387 | 0.0306 | 251 |
| 3 | 11 631 | 95 121 | 0.1222 | 502 |
| 4 | 22 206 | 93 256 | 0.2381 | 753 |
| 5 | 48 494 | 95 936 | 0.5054 | 1004 |
| 6 | 87 021 | 105 201 | 0.8271 | 1255 |
| 7 | 112 718 | 90 804 | 1.2413 | 1506 |
| 8 | 251 476 | 137 149 | 1.8335 | 1757 |
| 9 | 204 958 | 93 615 | 2.1893 | 2008 |
| 10 | 314 309 | 114 006 | 2.7569 | 2259 |
| 11 | 370 368 | 94 082 | 3.9366 | 2510 |

**Note.** $S_{DMSO}$: DMSO peak area; $S_{DES}$: DES peak area.

**TABLE 3.** Results of Chromatographic Analysis and Concentrations of Sulfur-Containing Substances in Some Drugs

| Drug, Company, lot | S-containing substance peak area, $cm^2$ | Internal standard (DES) peak area, $cm^2$ | Ratio of S-containing substance peak area to DES peak area | Concentration of S-containing substance in conversion to DMSO, μg/ml |
|---|---|---|---|---|
| Rheopolygluquin, Krasfarma Company (lot 2861204) | 7717 | 104 381 | 0.073931079 | 0.370 |
| Rheopolygluquin, Krasfarma Company (lot 440305) | 2632 | 95 081 | 0.027681661 | 0.229 |
| Rheopolygluquin, Belmedpreparaty Company (lot 2090902) | 24 343 | 83 981 | 0.289863183 | 0.727 |
| Rheopolygluquin, Biokhimik Company (lot 2911002) | 7073 | 107 115 | 0.066031835 | 0.350 |
| Rheopolygluquin, Biokhimik Company (lot 2340803) | 15 372 | 90 914 | 0.16908287 | 0.557 |

concentration of sulfur-containing substances was evaluated by the calibration curve or its equation.

The results of chromatographic analysis of the test specimens of some drugs and concentrations of volatile sulfur-containing substances are presented in Table 3.

According to requirements of the European Pharmacopoeia (5th version), the content of sulfur-containing substances released by rubber caps of $20\pm2$ $cm^2$ area should not exceed 22 μg in conversion to hydrogen sulfide. The actual area of the cap in contact with the contents of the bottle is about 6 $cm^2$. If the volume of the drug in a package is 400 ml, the content of sulfur-containing substances should not exceed 64 ng/ml (32 and 16 ng/ml for 200 and 100 ml dosages, respectively).