



THE METHOD OF QUANTITATION OF TRACES OF SOLVENTS IN PEPTIDES (C.26.1)

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Introduction:

The peptide is dissolved in water or Dimethylsulfoxide free of traces of solvent. The solvents are separated by capillary gas chromatography. The procedure follows closely the method of USP23 467 Determination of Organic Volatile Impurities.

Advantage:

- o Almost any solvent can be determined quantitatively.
- o Only about 0.5mg of the sample is required for this analysis.

Sample preparation:

Two samples are prepared:

- About 1,0 mg of sample is dissolved in 50 µl solvent free DMSO containing 2000ppm Dioxane. The sample is shaken vigorously for a minute using a Vortex shaker.
- The reference solution contains 200 ppm 1000 ppm of the solvents to be determined in 50 µL of solvent-free DMSO containing 2000 ppm of dioxane.
- 50 µL of solvent-free DMSO containing 2000 ppm of dioxane (internal standard solution).

The first sample is injected to calculate the content of the solvents using the internal standard method, the second to determine the retention times and/or response factors, the third to verify that the solvents are not present in the internal standard solution.



Chromatography:

These samples are injected directly into the GC using split injection.

The separation is performed by capillary gas chromatography using 20m*0.31mm Cyanopropylphenyl-Methyl- Silicon (1,8 µm film thickness) capillary Flame ionization detection.

The Carrier gas: Hydrogen
Flows: Carrier gas 1,5ml/min, Split: 35ml/min, Purge 4ml/min
Temperatures: Injector: 190°C; Detector 230°C;
Oven temperature: 55°C isotherm for 3 min, 5°C/min to 150°C,
20°C/min to 250°C

The peaks are identified by their retention times.

Each sample is analyzed twice if OOS is observed or in the case of GMP products. Quantitation is done by the internal standard method (2000ppm Dioxane).

Apparatus:

Gas chromatograph: Siemens SiChromat 2, Shimadzu17A or 2010.
Integrator: ChromPerfect Spirit
Vials, closures and heat block: C.A.T. H250, H500 and H103



Limit of quantitation/detection and standard deviation of the analysis:

Solvent	LOQ/LOD ppm at 1mg sample weight	Standard deviation at concentration 200 ppm
1,1,1-Trichloroethane	60/40	± 6%
Acetone	40/10	± 5%
Acetonitrile	40/10	± 5%
Benzene	30/5	± 5%
Butanol	40/10	± 6%
Chloroform	80/20	± 6%
dichloromethane	50/30	± 6%
Diethyl ether	30/10	± 5%
Diisopropyl amine	80/20	± 12%
Diisopropyl ether	50/10	± 5%
Diisopropylcarbodiimide	1000/400	± 8%
Diisopropylethylamine	200/100	± 12%
Dimethylacetamide	60/20	± 12%
Dimethylformamide	140/120	± 12%
Dimethylsulfoxide	500/100	± 12%
Ethanol	40/10	± 6%
Ethyl acetate	20/10	± 5%
Heptane	40/25	± 5%
i-Propanol	30/20	± 6%
Isohexane	80/25	± 5%
Isopropyl acetate	20/10	± 5%
Methanol	70/50	± 6%
Morpholine	90/60	± 12%
N-Methylmorpholine	30/20	± 12%
N-Methylpyrrolidinone	80/30	± 12%
Propanol	20/10	± 6%
t-Butanol	100/20	± 6%
Tetrachloromethane	80/80	± 6%
Toluene	20/5	± 5%
Triethylamine	150/50	± 12%
Tetrahydrofurane	20/10	± 6%



Calculation:

$$RF_i = \frac{\text{Amount of Component}}{\text{Area of Component}} * \frac{\text{Area Internal Standard}}{\text{Amount of Internal Standard}}$$

$$\text{Concentration}_i = \frac{RF_i * \text{Area}_i}{\sum_{i=1}^n RF_i * \text{Area}_i} * \frac{\text{Amount of Internal Standard}}{\text{Amount of Sample}}$$