PT SCHEME – 19 ROUND
TEST TASK NO. 2
QUANTITATIVE DETERMINATION OF ANALYTE IN SOLUTION USING LIQUID CHROMATOGRAPHY

The purpose of the test is an integral assessment of the correctness of conducting quantitative tests by the method of liquid chromatography (the SPhU, 2.2.29). The participant of the round is provided with two test solutions (TI) of metronidazole, which simulate the finished preparation with a nominal concentration of 0.3 mg/ml, and a method of quantitative determination. The laboratory's conclusion regarding compliance of the metronidazole content in the test solution with the specification (± 5% of the nominal value) is evaluated.

The content of metronidazole in the test solutions is such that, provided the laboratory works in accordance with the requirements of the SPhU (requirements of normal analytical practice for the uncertainty of sample preparation and requirements for the suitability of the chromatographic system), the laboratory with a probability of 99% should make a correct conclusion regarding the compliance of the content of the analyte with the specification.

Sample delivery kit: Test solution 1 (20 ml), Test solution 2 (20 ml), *CRS SPhU Metronidazole* (0.15 g), *CRS SPhU Metronidazole impurity A* (0.005 g).

Storage conditions: Test solution 1 and Test solution 2 are stored at temperatures from 2 °C to 8 °C. CRS SPhU is kept as stated in the certificate.

In order to enable preliminary tests on the suitability of the chromatographic system, *CRS SPhU Metronidazole* is supplied in a quantity sufficient to prepare two reference solutions (a).

Test samples for the task "Quantitative determination of analyte in solution by liquid chromatography" are provided in the amount for one test. Additional samples for testing by more than one analyst may be purchased by the laboratory for an additional fee.

Metronidazole quantitative determination procedure.

Liquid chromatography (2.2.29).

Test solution. Place 10.0 mL of Metronidazole test solution in a 100 mL volumetric flask, add 20 ml of *methanol P*, dilute to volume with *water P*. The shelf life of the solution is 24 hours.

Reference solution (a). Place 75.0 mg of CRS SPhU Metronidazole in a volumetric flask 250 mL, dissolve in 80 ml of *water P*, dilute to volume with the same solvent. The shelf life of the solution is 48 hours.

Reference solution (b). Place 10.0 ml of reference solution (a) in a 100 ml volumetric flask, add 20 mL of *methanol P*, dilute to volume with *water P*. The shelf life of the solution is 24 hours.

Reference solution (c). 5.0 mg of *CRS SPhU Metronidazole impurity A* is placed in a 50 mL volumetric flask, dissolved in the mobile phase and dilute to volume with the same solvent. The shelf life of the solution is 62 hours.

Place 2.0 ml of the obtained solution in a 100 mL volumetric flask, add 0.35 mL of the *reference solution* (a), dilute to the volume with the mobile phase. The shelf life of the solution is 24 hours.

Column:
— size: 0.15 m × 4.6 mm;
— stationary phase: *silica gel for chromatography octylsilyl P* (5 μm);
— температура: 30 °C.

Mobile phase: methanol R, *water R* (20 : 80);

Flow rate: 1.0 *ml/min*.

Detection: spectrophotometric at a wavelength of 319 nm.

Injection: 30 μL.

Chromatograph the following solutions: reference solution (b), reference solution (c), test solution.

Suitability of the chromatographic system:
reference solution (c):

— *resolution*: not less than 4.0 between the peaks of metronidazole and metronidazole impurity A;
reference solution *(b)*:
— *symmetry factor*: not more than 2.0;
— *precision*: accordance with the requirements *2.2.46*^{N} (also in: WHO good practices for pharmaceutical quality control laboratories. WHO TRS 57, Annex 4, Appendix 2. https://iris.who.int/bitstream/handle/10665/376607/9789240091030-eng.pdf)

Limits:
— content of $C_6H_9N_3O_3$ in the test solution not less than 0.285 mg/mL and not more than 0.315 mg/mL.

Calculate the content of $C_6H_9N_3O_3$ in test solution 1 and test solution 2, in mg/mL, based on the declared content of $C_6H_9N_3O_3$ in *CRS SPhU metronidazole*. The conformity of the content of metronidazole in the test solution with the requirements of the specification is assessed.

Test results are recorded in the report form.

PT SCHEME – 19 ROUND
RESULTS OF EXECUTION OF TEST TASK NO. 2

QUANTITATIVE DETERMINATION OF THE ANALYTE IN SOLUTION BY THE LC METHOD

Name of the laboratory	
The head of the laboratory	
Analyst	
Date of receipt of test solutions (TI)	
The date of the analysis	

The equipment used:

Balance			
Balance model and manufacturer			
Balance parameters according to the passport	Resolution, mg	Precision, mg	Linearity, mg
Date of metrological verification			
Was the balance qualification carried out?	□ Yes	(date)	□ No
If "Yes": qualification parameters and acceptance criteria	Parameter	Acceptance criteria	Value
	…	…	…
Volumetric glassware			
What standard do the volumetric flasks used correspond to?	_____ *(specify the number of the standard)*		
Volumetric flasks of which class were used?	□ Class A		□ Class B
Was verification of volumetric flasks carried out??	□ Yes		□ No
If "so", indicate the procedures carried out:			
– visual inspection	□ Yes _____ (date)		□ No
– analytical evaluation (calibration)	□ Yes _____ (date)		□ No
What standard do the pipette(s) used to prepare the test solutions TP1, TP2 correspond to?	_____ *(specify the number of the standard)*		
Pipette(s) of what class were used?	□ Class A		□ Class B
Was the pipette(s) verified?	□ Yes		□ Hi
If "so", indicate the procedures carried out:			
– visual inspection	□ Yes _____ (date)		□ No
– analytical evaluation (calibration)	□ Yes _____ (date)		□ No
Chromatograph			
Chromatograph type and brand			
Date of metrological verification			
Has the chromatographic system been qualified?	□ Yes _____ (date)		□ No
If "so": 1) Qualification parameters and criteria of acceptance	Parameter	Value	Acceptance criteria
	...	…	...
2) On the basis of which document the qualification program was drawn up?			
Column name, sorbent type, geometric parameters, particle size, etc			

Test results:

Preparation of solutions	
Reference solution (a) Test portion of *CRS SPhU Metronidazole*, mg	
Reference solution (c) Test portion of *CRS SPhU Metronidazole impurity A*, mg	

The sequence of obtaining chromatograms (experimental design)	
Total number of chromatograms, pcs.	
Reference solution (c), номери хроматограм	N …
Reference solution (b), номери хроматограм	N …
Test solution (TP1), номери хроматограм	N …
Test solution (TP2), номери хроматограм	N …

Checking the suitability of the chromatographic system		
Resolution*	Symmetry factor*	Relative standard deviation (RSD), %

* In case of obtaining several values of the parameter, the worst value is given in the table.

Chromatography results of the tested solution (TP1)		
Retention time of the main peak, min	Peak area	The peak area average value
…	…	

Chromatography results of the tested solution (TP2)		
Retention time of the main peak, min	Peak area	The peak area average value
…	…	

Chromatography results of the reference solution (b)		
Retention time of the main peak, min	Peak area	The peak area average value
…	…	

Calculation results, conclusions		
Test solution	Content of $C_6H_9N_3O_3$, mg/mL	Compliance with the specification
TP1		☐ comply ☐ not comply
TP2		☐ comply ☐ not comply

The head of the laboratory _____/_____
(signature)