กรมวิทยาศาสตร์การแพทย์
Department of Medical Sciences

PROFICIENCY TESTING PROGRAM 2023

Scheme Code D6601R1

Assay by HPLC

PROFICIENCY TESTING PROTOCOL

No. D6601R1

Organized by

Bureau of Drug and Narcotic
Department of Medical Sciences
88/7 Soi Bumrasnaradura Hospital, Tiwanon Road, Nonthaburi, 11000
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1. ORGANIZER

Scheme Provider:

Bureau of Drug and Narcotic (BDN)
Department of Medical Sciences
88/7 Soi Bumrasnaradura Hospital, Tiwanon Road, Nonthaburi,
11000, THAILAND
Tel. +66 2951 0000 ext. 99112
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Scheme Coordinator:

Ms. Supawadee Surangkul
Pharmacist, Senior professional level
Email: pt_bdn@hotmail.com; pts.bdn@gmail.com
The scheme coordinator is responsible for all activities of the PT scheme.

2. OBJECTIVE

The objective of this Proficiency Testing (PT) scheme is to demonstrate that participants can accurately perform assay by using High Performance Liquid Chromatography (HPLC) technique. The scheme is based on the analysis of pharmaceutical substance, acetylcysteine, according to the method from the United States Pharmacopeia (USP) 2022 in Acetylcysteine monograph and General Chapters: <621> Chromatography.

3. SUBCONTRACT

BDN has no policy to subcontract in any activity of this proficiency testing scheme.

4. CRITERIA FOR PARTICIPATION

Participants should have competency to perform assay of pharmaceutical substance by HPLC and use this technique in their routine work. Each participant is able to participate in the program for one set of PT sample.

5. NUMBER AND TYPE OF PARTICIPANTS

Number of participants: 20 – 80 laboratories

Type of participants: Quality control laboratories in pharmaceutical manufacturers and other testing laboratories

6. SCHEDULE OF PT SCHEME

The frequency of this PT program is at least one round per 2 years. Schedule for the PT activities is as follows:

Activity	Schedule
Call for participation	December 2022
Deadline for registration	31 January 2023
Distribution of samples	April 2023
Deadline for submission of results	31 May 2023
Interim report for comments	June 2023
Final report	July 2023

For the best efficiency of PT program, all activities will be operated according to this time frame. Participants are requested to feedback for registration and submission of result according to the schedule. Participants should note that results submitted are not allowed to change or amend in any case.

7. REGISTRATION

Registration Fee:

Local participants	5,000.- Baht
Oversea participants	200.- USD (Including shipping handling)

Payment:

***Local participants*-**

The payment should be made by Teller payment system at any branches of Krung Thai Bank Public Company Limited.

***Overseas participants*-**

The payment should be made by bank transfer to

Account name:	DMSc Non-Fiscal Budget Account
Account number:	1301-034924
Bank name:	Krung Thai Bank Public Company Limited
Branch address:	88/20 1st Floor Block E Ministry of Public Health Moo 4 Soi Bamrat Naradul, Tiwanon Road, Talat Khwan, Mueang Nonthaburi, Nonthaburi, 11000
Swift code:	KRTHTHBK

Method and Deadline for Registration:

Participants should register online via the website **https://www.bdn.go.th/pt** within ***31 January 2023***. Registration by other means e.g. fax, Email will not be accepted.

Terms and Conditions:

1. BDN reserves the right to occasionally delay the issue of PT program or use an appropriate substitute test material with prior warning to participants if the planned PT sample is not available according to the schedule of PT scheme.
2. The registration fees are nonrefundable.
3. Charge or fee of financial transactions is the responsibility of participant.

More information can be viewed on the website https://www.bdn.go.th/pt.

8. PROFICIENCY TESTING SAMPLE

The sample of this scheme is pharmaceutical substance, Acetylcysteine.

9. HANDLING OF PT SAMPLE

Participants will receive (i) a bottle of Acetylcysteine sample for PT containing about 850 mg. (ii) a bottle of Acetylcysteine reference standard for PT containing about 250 mg. (iii) a bottle of L-Phenylanalanine containing about 125 mg. and (iv) a bottle of Sodium Metabisulfite containing about 1 g.

PT samples, Acetylcysteine reference standard and L-Phenylanalanine are packed in 4-ml amber glass bottle with plastic screw cap and should be stored between 2-8°C and protected from light and humidity until analysis. Sodium Metabisulphite is packed in 4-ml amber glass bottle with plastic screw cap and should be stored below 30°C. All of them are labeled which show the details of name, amount and storage condition and packed in vacuum plastic package. They are distributed in ambient condition.

PT samples are sent by express mail for local participants and by courier for overseas participants. It is the responsibility of the participants to contact BDN as soon as possible if they have not received the PT sample within the time schedule. In case of international transport for overseas participants, BDN cannot be responsible for any delays from the custom clearance. Upon receipt of PT sample, participants are requested to check physical conditions of PT sample as well as other substances provided and complete a PT sample acknowledgement form via the website https://www.bdn.go.th/pt.

10. TEST METHOD

Participants should determine in triplicates the percentage content of acetylcysteine ($C_5H_9NO_3S$) and report the results with **one digit after the decimal separator**. In case number of digit reported is different from that specified in the protocol, the results will be rounded to one digit by BDN. If the right of the last decimal place is smaller than 5, it is eliminated and the preceding digit is unchanged. If the right of the last decimal place is equal to or greater than 5, it is eliminated and the preceding digit is increased by 1. Results reported with no digit after decimal separator or less than that specified in the protocol, 0 will be substituted. Range of percentage of labeled amount for this PT sample is 97.0-103.0%.

Analysis is based on the assay method specified in Acetylcysteine monograph of USP 2022 using HPLC according to the following procedure.

Procedure:
Mobile phase: 6.8 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0.
Sodium metabisulfite solution: 0.5 mg/mL of sodium metabisulfite in water, freshly prepared
Internal standard solution: 5 mg/mL of L-Phenylalanine in sodium metabisulfite solution
Standard stock solution: 10 mg/mL of Acetylcysteine reference standard in sodium metabisulfite solution
Standard solution:0.5 mg/mL of Acetylcysteine reference standard and 0.25 mg/mL of L-Phenylalanine in sodium metabisulfite solution from standard stock solution and internal standard solution
Sample stock solution: 10 mg/mL of Acetylcysteine in sodium metabisulfite solution
Sample solution: 0.5 mg/mL of Acetylcysteine and 0.25 mg/mL of L-Phenylalanine in sodium metabisulfite solution from sample stock solution and internal standard solution
Chromatographic system
(See Chromatography ⟨621⟩, System Suitability.)
Mode: LC
Detector: UV 214 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1.5 mL/min
Injection size: 5 µL
System suitability
Sample: Standard solution
[NOTE—The relative retention times for Acetylcysteine and L-Phenylalanine are about 0.5 and 1.0, respectively.]

Suitability requirements
Resolution: NLT 6 between Acetylcysteine and L-Phenylalanine
Relative standard deviation: NMT 2.0%

Analysis
Samples: Standard solution and sample solution

Details of the method are described in the testing protocol which can be downloaded via Email from website https://www.bdn.go.th/pt after sample distribution. PT sample as well as analytical procedure should be handled and operated in the same manner as performing routine work.

11. ASSIGNED VALUE

The assigned value used to calculate z score is based on the consensus value from participants using the robust mean calculated according to robust analysis: Algorithm A in Annex C of ISO 13528:2015 (E)-Statistical methods for use in proficiency testing by interlaboratory comparisons. The assigned value will not be disclosed to participants until interim report is issued.

The standard uncertainty of the assigned value x_{pt} is estimated as

$$u(x_{pt}) = 1.25 \times \frac{s^*}{\sqrt{p}}$$

Criteria:
If

$$u(x_{pt}) \quad < \quad 0.3\, \sigma_{pt}$$

then, the uncertainty of the assigned value is negligible and need not to be included in the interpretation of the results.

12. STANDARD DEVIATION FOR PROFICIENCY ASSESSMENT

Standard deviation for proficiency assessment used to calculate z scores is set as 1.0% that corresponds to the level of performance and the expected precision of the test method or techniques and according to fitness for purpose.

13. PERFORMANCE EVALUATION

Participants will be assessed on the differences between their results and the assigned value. The z score is used for the performance evaluation as

$$z_i = \frac{(x_i - x_{pt})}{\sigma_{pt}}$$

z_i = z score

x_i = measurement result from participant

x_{pt} = assigned value

σ_{pt} = standard deviation for proficiency assessment

The interpretation of z score is designated as follows.

$\|z\| \leq 2.0$	: acceptable
$2.0 < \|z\| < 3.0$	: warning signal
$\|z\| \geq 3.0$	: unacceptable

When $u(x_{pt}) > 0.3\sigma_{pt}$, then the uncertainty can be taken into account by expanding the denominator of the performance score and calculated as *ź* score

$$z_i' = \frac{x_i - x_{pt}}{\sqrt{\sigma_{pt}^2 + u^2(x_{pt})}}$$

The interpretation of z'score is as same as z score and using the same critical values of 2.0 and 3.0, depending on the design for the PT scheme.

For warning signal or unacceptable results, it is recommended that participants should investigate root causes and take necessary corrective actions.

14. POTENTIAL MAJOR SOURCES OF ERRORS

1. Suitability of analytical balance used and weighing procedure
2. Accuracy of sample and reference standard solution preparation
3. Suitability of chromatographic system
4. Calculation of the result
5. Moisture uptake due to inappropriate sample handling

15. REPORT

An interim report is issued to provide each participant with an early indication of performance. In general, the interim report is issued about two months after the deadline for submission of results via the website https://www.bdn.go.th/pt. Participants are requested to check and review for any correction and/or comment. The final report is issued via the website https://www.bdn.go.th/pt after the correction and/or comment of interim report has been completed.

The report includes the following information.

- Introduction: general description of PT scheme
- Name and contact details of proficiency testing provider and scheme coordinator
- Participation: information of participating laboratories
- PT sample: description, sample preparation
- Homogeneity and stability assessment
- Assigned value, including measurement uncertainty and standard deviation of proficiency assessment
- Results: result tables including statistic summary data, z scores and bar chart of z scores
- Discussion of results: conclusion of overall performance and comments on Participant's performance
- Potential major sources of errors

16. CONFIDENTIALITY, COLLUSION AND FALSIFICATION OF RESULTS

The identities of participants are protected by means of laboratory codes which are randomly assigned in each PT round. These codes are confidential and are not disclosed to other persons unless agreed by the participant for a regulatory or recognition purpose. Participants can access their personal information in the website https://www.bdn.go.th/pt by using username and password which can keep their information confidentially. For security purpose, participants are recommended to change username and password in case of changing the responsible person who can access the information in the website.

This PT scheme is conducted in the belief that participants will perform the analysis and report results with scientific professional. Where any collusion between participants or falsification of results is proven by BDN, the result of that participant for the PT round concerned will be cancelled for performance evaluation.

17. LOST OR DAMAGED OF PROFICIENCY TESTING SAMPLE

Email will be sent to inform participants after sample distribution. In case of lost or damaged PT sample, participants should immediately inform BDN via https://www.bdn.go.th/pt. In some cases, the damaged PT sample should be returned to BDN. Replacement will be arranged within 1 week if the PT samples are proved to be lost or not suitable for analysis. The deadline of submitting result will be extended to appropriate date if necessary.