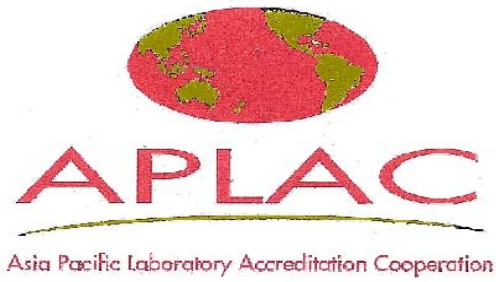


Pharmaceutical Preparation Analysis

Proficiency Testing Program
APLAC T080

FINAL REPORT

1 November 2011

CONTENTS

Summary of Results

1. The proficiency testing program (APLAC T080) aimed at evaluating the performance of laboratories for determination of active pharmaceutical ingredients in pharmaceutical preparation by HPLC.

2. A total of 38 laboratories from 18 economies enrolled in the program and 33 of them returned results to the organizers.

3. The assigned values (robust averages) and standard deviations (robust standard deviations) for performance assessment are summarized as follows:

	Hydrochlorothiazide	Captopril
Robust Average	7.220	12.39
Robust Standard Deviation(σ_R)	0.152	0.198

4. The z-score was used as the numerical indicator to show participants' performance with respect to the assigned values in the program. The z-scores of hydrochlorothiazide and captopril are summarized as follows:

z-Score	**Number of Participants (Percentage)**	
	Hydrochlorothiazide	**Captopril**
$\|z\| \leq 2$	27 (82%)	29 (88%)
$2 < \|z\| < 3$	5 (15%)	1 (3%)
$\|z\| \geq 3$	1 (3%)	3 (9%)
Total:	33 (100%)	33 (100%)

1. Introduction

1.1. The combination of captopril and hydrochlorothiazide is used to treat high blood pressure. Captopril is in a class of medications called angiotensin-converting enzyme (ACE) inhibitors. It works by decreasing certain chemicals that tighten the blood vessels, so blood flows more smoothly. Hydrochlorothiazide is in a class of medications called diuretics. It works by causing the kidneys to get rid of unneeded water and salt from the body into the urine.

1.2. A proficiency testing program (APLAC T080) on determining the contents of hydrochlorothiazide and captopril in pharmaceutical preparation by HPLC was organised by Shanghai Institute for Food and Drug Control(SIFDC), in collaboration with China National Accreditation Service for Conformity Assessment(CNAS), and under the auspices of the Asia-Pacific Laboratory Accreditation Co-operation (APLAC). The program was approved by APLAC PT Committee in April 2011 and was expected to be completed in December 2011 (TABLE I).

1.3. A total of 38 laboratories from 18 economies enrolled in the program and 33 of them returned their results on or before the deadline (TABLE II).Participants were confidentially assigned with a unique laboratory code (Lab 001 to lab 038) and the code was used throughout the program.

2. Objectives

2.1. Pharmaceutical preparation plays an important role in APEC trade and domestic markets. The content of active pharmaceutical ingredients affects the safety and effectiveness of medication significantly. The objective of this proficiency testing program is to evaluate the performance of laboratories for determination of active pharmaceutical ingredients in pharmaceutical preparation.

2.2. The robust averages and robust standard deviations for performance

assessment were calculated using the consensus value from participants (ISO 13528: 2005 Statistical Methods for Use in Proficiency Testing by Inter-laboratory Comparisons Annex C Robust analysis: Algorithm A) [8.1].

3. Test Material

3.1. Captopril, Hydrochlorothiazide and suitable excipients were collected from a pharmaceutical company in China. The materials were ground to fine powder separately and then thoroughly mixed in high speed blender. Homogenized powder was then packed into brown glass bottles. More than 300 bottles of samples were finally prepared and stored at a temperature of below 30℃ before shipment.

3.2. The homogeneity and stability of samples were processed and checked in accordance with Method in ISO 13528: 2005 Annex B. The tests for sample homogeneity (performed in June 2011) and stability (July 2011 and October 2011) were carried out in accordance with the procedures stipulated in APPENDIX II and III. Results indicated the testing materials are suitable to be used in the proficiency testing program.

3.3. The tests for the LOD of the samples were performed at June 2011 and October 2011. The results of the t-test stipulated in APPENDIX IV indicated that there was no significent difference in the period. It means the sealed condition was fine.

3.4. Each participant was provided with ONE brown glass bottle, labeled as “APLAC T080 XXX” which contains about 2 g of white powder (equivalent to powdered tablets), from the organizers or via their respective accreditation bodies (ABs). The samples were dispatched by EMS on 10th August 2011. Relevant documents included “Instruction”, “Receipt Form” and “Results Proforma” were also provided via emails at the time of dispatch. A specimen copy of these instructions was illustrated in APPENDIX IV to VIII.

3.5. Upon receipt of the test material, participants were requested to check the physical conditions of the bottle and promptly acknowledge the organizers by returning the “Receipt Form” to SIFDC at **aplac_t080@sina.com**. Replacement would be arranged if any defect was detected. The organizers received no abnormality report in this program.

4. Test Required

4.1. Participants were required to use suitable HPLC methods (could be pharmacopoeia and in-house methods, etc.) to determine the contents of hydrochlorothiazide and captopril.

4.2 Intact sample is recommended to be stored in a secure environment at a temperature of below 30°C before use. Once the sample bottle is opened, the test must be performed immediately.

4.3 Participants were required to report the result of LOD (Dry about 1 g sample in vacuum at 60°C for 3 hours). This is for the organizors to monitor the sealed condition of sample bottles.

4.4. All the analytical results and information should be reported in the Results Proforma provided.

5. Statistical Evaluation

5.1. Performance evaluation

5.1.1. Performance of participants was assessed using z-score, which is calculated as:

$Z = (Xi - X)/ \sigma_R$

Where Xi - Reported result from individual participant

X - Robust average

σ_R - Robust standard deviation

5.1.2. z-Score is interpreted as follows:

(a) $|z| \leq 2$ Satisfactory

(b) $2 < |z| < 3$ Questionable

(c) $|z| \geq 3$ Unsatisfactory

5.1.3. Participants having z-score(s) ≥ 3 or ≤ -3 should thoroughly investigate their results and report the findings to their AB. Participants having z-score(s) in the range $2 < |z| < 3$ are encouraged to review their results.

5.2. An interim report containing participants' data and the respective z-score results was issued to participants and their ABs on 30 September 2011. Participants were requested to check the correctness of their submitted data and to inform the organizers of any mistakes. The organizer received the results of Lab. 022 after the issurance of the interim report because that the lab sent it to a wrong e-mail address.

6. Results and Discussions

6.1. Test results and the technical information of methods used by participants are presented in TABLE III to TABLE VII.

6.2. Number of participants' data submitted is summarized as follows:

	Hydrochlorothiazide	Captopril
No. of numeric data	33	33

6.3. Assigned values and standard deviations for performance assessment

6.3.1. The assigned values and standard deviations for performance assessment

were calculated using the consensus value from participants (ISO 13528: 2005 Annex C Robust analysis: Algorithm A).

6.3.2. Assigned values and standard deviations:

	hydrochlorothiazide	captopril
Assigned value (%)	7.220	12.39
Standard deviation (σ_R)	0.152	0.198

6.4. An overview of participants' results

6.4.1. Participants' z-scores for the hydrochlorothiazide and captopril are presented in TABLE V. As illustrated in FIGURE I (a) to II (a). These reported mean concentrations, in ascending order, for all participants are shown in FIGURE I (b) to II (b).

6.4.2. Laboratories who did not report the values of contents to 4 significant figures in accordance with the provisions of note 1 in RESULTS PROFORMA are listed as follw.This is worthy of attention for these laboratories.

Component	Lab Code
Hydrochlorothiazide	Lab.004 Lab.005 Lab.006 Lab.008 Lab.012 Lab.017 Lab.023 Lab.025 Lab.032 Lab.037
Captopril	Lab.001 Lab.004 Lab.023 Lab.024 Lab.025 Lab.032 Lab.036 Lab.037

6.4.3. Performance of participants, in terms of z-score, is summarized as follow:

z-Score	Number of Participants (Percentage)	
	Hydrochlorothiazide	Captopril
$\|z\| \leq 2$	27 (82%)	29 (88%)
$2 < \|z\| < 3$	5 (15%)	1 (3%)
$\|z\| \geq 3$	1 (3%)	3 (9%)
Total:	33 (100%)	33 (100%)

In brief, 4 participants (Lab Code 004, 006, 020, 036) were identified as having reported at least one unsatisfactory results; and 4 out of the 66 results (6.1%) submitted were identified as unsatisfactory results.

6.4.4. The Z-scores of Lab.017 are 1.91 for hydrochlorothiazide and 1.77 for captopril. But the results' relative deviations of this laboratory for hydrochlorothiazide and captopril in duplicate are both too large. The organizer suggests Lab.017 to investigate to determine the cause and take suitable action.

6.5. Instrumental methods

6.5.1. HPLC-UV is a popular method for determination of contents of active pharmaceutical ingredients than other methods. As shown in TABLE V, all of the participants used HPLC-UV (33 laboratories) for the analysis. Eighteen of them were equipped with common reversed phase columns (such as C18 or C8), thirteen of them were equipped with phenyl columns, and the other two participants didn't provide the information of columns.

6.5.2. No significant difference of theoretical plate between common reversed phase columns and phenyl columns is observed, nor significant difference of resolution either. But as shown in TABLE VI and VII, 3 out of all 4 participants who gaved unsatisified results used the phenyl columns. But no special reason indicates that the

phenyl columns lead to the unsatisified results.

Best/worst	Theoretical plate		Resolution
	Hydrochlorothiazide	Captopril	
C18,C8 Column	23506/2000	20417/646	23.9/2.19
phenyl Column	49174/2078	10625/728	11.9/2

6.6. Accreditation Status versus Performance

6.6.1. twenty-nine participants claimed their methods are accredited. Only four do not have the accreditation status.

6.6.2. There is significant difference in performance between the accredited laboratories and the non-accredited counterparts in this program. Three out of fifty-eight data (or 5%) from the accredited laboratories were identified as unsatisfactory, while there are two out of eight (or 25%) from the non-accredited laboratories.

7. Remarks

7.1. Contributions from all ABs and participants to this program are gratefully noted. Special thanks are also extended to APLAC PT Committee, CNAS and Shanghai Food and Drug Administration for their support to the program.

7.2. For further comments or disagreement with the report, please promptly contact the organizers by mail or email:

Yang Mei-cheng
1500, Zhangheng Rd, Pudong New Area.
Shanghai Institute for Food and Drug Control

email: aplac_t080@sina.com

7.3. The organizer (SIFDC) is not accredited for providing those technical commentaries listed in Section 6.

7.4. Use of this report by participants shall only be allowed with the written permission of the program's organizers.

8. References

8.1. ISO 13528:2005. Statistical methods for use in proficiency testing by interlaboratory comparisons, 2005, Geneva, Switzerland.

TABLE I: Program Timeline

EVENT	PERIOD
Submission of proposal to APLAC PT committee	Nov 2010
Preparation of sample	Apr-May 2011
Homogeneity testing	Jun 2011
Stability testing	Jul-Oct 2011
Invitation to participants	Jun-Jul 2011
Dispatch of samples	Aug 2011
Submission of results	Sep 2011
Statistical analysis of results	Sep 2011
Interim report	Sep 2011
Submission of draft report to APLAC PT Committee	Nov 2011
Approval of draft report by APLAC PT Committee	Dec 2011
Distribution of final report	Dec 2011

TABLE II: Geographical Distribution of Participants

No.	ECONOMIES	PARTICIPANTS ENROLLED	RETURNED RESULTS
1	Australia	1	1
2	Belgium	2	2
3	Canada	1	1
4	China	6	6
5	Colombia	1	1
6	Greece	2	2
7	Hongkong	2	2
8	India	4	2
9	Indonesia	4	4
10	Ireland	1	1
11	Israel	1	1
12	Kazakhstan	2	2
13	New Zealand	2	2
14	Portugal	1	1
15	Singapore	1	1
16	Spain	1	1
17	Switzerland	2	2
18	USA	4	1
	Total:	38	33

TABLE III: Participants' Results for Hydrochlorothiazide

Lab Code	Concentration(%)		
	Data #1	Data #2	Mean
LAB. 001	7.187	7.116	7.151
LAB. 002	7.109	7.107	7.108
LAB. 003	7.229	7.221	7.225
LAB. 004	6.8409	6.6468	6.7438
LAB. 005	6.92	6.82	6.87
LAB. 006	7	7.12	7.06
LAB. 007	7.168	7.129	7.148
LAB. 008	7.21	7.24	7.23
LAB. 009	7.576	7.487	7.532
LAB. 010	7.545	7.531	7.538
LAB. 011	7.140	7.105	7.123
LAB. 012	7.18	7.11	7.14
LAB. 013	7.176	7.177	7.176
LAB. 014	7.222	7.272	7.247
LAB. 015	***	***	***
LAB. 016	---	---	---
LAB. 017	7.78	7.23	7.51
LAB. 018	---	---	---
LAB. 019	7.247	7.187	7.217
LAB. 020	7.480	7.480	7.480

TABLE III: Participants' Results for Hydrochlorothiazide(Cont'd)

Lab Code	Concentration(%)		
	Data #1	Data #2	Mean
LAB. 021	7.342	7.364	7.353
LAB. 022	7.196	6.964	7.080
LAB. 023	7.2959	7.3220	7.3089
LAB. 024	7.590	7.595	7.59
LAB. 025	7.7710	7.5411	7.6560
LAB. 026	7.228	7.343	7.286
LAB. 027	7.161	7.131	7.146
LAB. 028	7.162	7.136	7.149
LAB. 029	7.207	7.191	7.199
LAB. 030	7.238	7.241	7.240
LAB. 031	7.104	7.084	7.094
LAB. 032	7.2039	7.1681	7.186
LAB. 033	7.224	7.226	7.225
LAB. 034	---	---	---
LAB. 035	***	***	***
LAB. 036	7.304	7.314	7.309
LAB. 037	7.1639	7.1641	7.1640
LAB. 038	7.067	7.056	7.062

"***" denotes data/information was not provided by participants
"---" denotes the participant quited form the program

TABLE IV: Participants' Results for Captopril

Lab Code	Concentration(%)		
	Data #1	Data #2	Mean
LAB. 001	12.220	12.150	12.185
LAB. 002	12.51	12.49	12.50
LAB. 003	12.44	12.47	12.46
LAB. 004	12.3179	11.9630	12.1404
LAB. 005	12.22	11.90	12.06
LAB. 006	13.03	13.06	13.045
LAB. 007	12.60	12.62	12.61
LAB. 008	12.37	12.30	12.34
LAB. 009	12.17	12.25	12.21
LAB. 010	12.50	12.52	12.51
LAB. 011	12.21	12.24	12.23
LAB. 012	12.01	12.53	12.27
LAB. 013	12.44	12.49	12.46
LAB. 014	12.50	12.54	12.52
LAB. 015	***	***	***
LAB. 016	---	---	---
LAB. 017	13.24	12.24	12.74
LAB. 018	---	---	---
LAB. 019	12.37	12.30	12.33
LAB. 020	14.29	14.25	14.27

TABLE IV: Participants' Results for Captopril(Cont'd)

Lab Code	Concentration(%)		
	Data #1	Data #2	Mean
LAB. 021	12.43	12.36	12.39
LAB. 022	12.75	12.56	12.66
LAB. 023	12.4824	12.4964	12.4894
LAB. 024	12.365	12.360	12.36
LAB. 025	12.2159	12.3049	12.2604
LAB. 026	11.80	12.04	11.92
LAB. 027	12.38	12.39	12.39
LAB. 028	12.30	12.36	12.33
LAB. 029	12.33	12.31	12.32
LAB. 030	12.14	12.34	12.24
LAB. 031	12.38	12.29	12.34
LAB. 032	12.385	12.389	12.39
LAB. 033	12.43	12.44	12.44
LAB. 034	---	---	---
LAB. 035	***	***	***
LAB. 036	13.207	13.147	13.177
LAB. 037	12.2656	12.2840	12.2748
LAB. 038	12.25	12.25	12.25

"***" denotes data/information was not provided by participants
"---" denotes the participant quited form the program

TABLE V: z-Scores of Participants

Lab. Code	Hydrochlorothiazide	Captopril
LAB. 001	-0.45	-1.04
LAB. 002	-0.74	0.56
LAB. 003	0.03	0.35
LAB. 004	-3.13	-1.26
LAB. 005	-2.30	-1.67
LAB. 006	-1.05	3.31
LAB. 007	-0.47	1.11
LAB. 008	0.07	-0.25
LAB. 009	2.05	-0.91
LAB. 010	2.09	0.61
LAB. 011	-0.64	-0.81
LAB. 012	-0.53	-0.61
LAB. 013	-0.29	0.35
LAB. 014	0.18	0.66
LAB. 015	***	***
LAB. 016	---	---
LAB. 017	1.91	1.77
LAB. 018	---	---
LAB. 019	-0.02	-0.30
LAB. 020	1.24	9.49

TABLE V: z-Scores of Participants(Cont'd)

Lab. Code	Hydrochlorothiazide	Captopril
LAB. 021	0.88	-0.20
LAB. 022	-0.92	1.36
LAB. 023	0.58	0.50
LAB. 024	2.43	-0.15
LAB. 025	2.87	-0.65
LAB. 026	0.43	-2.37
LAB. 027	-0.49	0.00
LAB. 028	-0.47	-0.30
LAB. 029	-0.14	-0.35
LAB. 030	0.13	-0.76
LAB. 031	-0.83	-0.25
LAB. 032	-0.22	0.00
LAB. 033	0.03	0.25
LAB. 034	---	---
LAB. 035	***	***
LAB. 036	0.59	3.97
LAB. 037	-0.37	-0.58
LAB. 038	-1.04	-0.71

z-Scores ≥ 3 or ≤ -3 are underlined

"***" denotes data/information was not provided by participants

"---" denotes the participant quited form the program

TABLE VI: Instrumentation and Reference Information

Lab Code	Analytical Instrument	Analytical Column	Mobile Phase	Detective Wavelength	Reference
LAB. 001	HPLC-DAD	XBrigde C18 4.6×150mm	Phosphate buffer/ACN	/	Eu.P
LAB. 002	HPLC-UV	Waters symmetry C18 4.6×150mm	Phosphate buffer/ACN	210 nm	MAH
LAB. 003	HPLC-UV	Hypersil Gold 4.6×200 mm, 5μm Zorbax C18 Plus 4.6×250mm, 5μm	H_2O/MeOH/H_3PO_4 NaH_2PO_4 Buffer/ ACN	210 nm /254 nm	SOP
LAB. 004	HPLC-UV	Zorbax SB-Phenyl 4.6×250mm, 5μm	H_2O/MeOH/H_3PO_4	210 nm	USP
LAB. 005	HPLC-UV	Hypersil Phenyl 4.6×250mm, 5μm	H_2O/MeOH/H_3PO_4	210 nm	USP
LAB. 006	HPLC-UV	/	/	220nm	/
LAB. 007	HPLC-PDA	Inertsil Ph 4.6×250mm,5μm	H_2O/MeOH/H_3PO_4	210nm	USP
LAB. 008	HPLC-UV	Spherisorb Phenyl 4.6×250mm,5μm	H_2O/MeOH/H_3PO_4	210nm	/
LAB. 009	HPLC-UV	Phenomener Spherisorb C8 4.6×250mm, 5μm	H_2O/MeOH/H_3PO_4	210nm	USP
LAB. 010	HPLC-UV	Phenyl,hexyl 4.6×250mm, 5μm	H_2O/ACN/H_3PO_4	210nm	USP
LAB. 011	HPLC-DAD	Symmetry C18 4.6×250mm, 5μm	H_2O/MeOH/H_3PO_4	210nm	USP
LAB. 012	HPLC-UV	μBondapack C18 3.9×300mm 10μm	H_2O/MeOH/H_3PO_4	210nm	USP
LAB. 013	HPLC-UV	Lichrosorb C8 4.6×250mm ,10μm	phosphate buffer/ACN	215nm	SOP
LAB. 014	HPLC-UV	μBondapack C18 3.9×300mm 10μm	H_2O/ACN/H_3PO_4	210nm	USP
LAB. 017	HPLC-UV	/	H_2O/MeOH/H_3PO_4	210nm	USP
LAB. 019	HPLC-UV	C18 2.1×250mm, 5μm	/	/	USP
LAB. 020	HPLC-UV	Platinum Phenyl 4.6×250mm ,10μm	H_2O/MeOH/H_3PO_4	210nm	USP
LAB. 021	HPLC-UV	Hypersil C18 4.6×250mmmm, 5μm	H_2O/MeOH/H_3PO_4 H_2O/ACN/H_3PO_4	220nm/254nm	USP/BP

TABLE VI: Instrumentation and Reference Information(Cont'd)

Lab Code	Analytical Instrument	Analytical Column	Mobile Phase	Detective Wavelength	Reference
LAB. 022	HPLC-UV	Luna C18 4.6×250mmmm, 5μm	H_2O/MeOH/H_3PO_4	210nm	USP
LAB. 023	HPLC-UV	CLC-Phenyl 6×150mm, 5μm	H_2O/MeOH/H_3PO_4	210nm	USP
LAB. 024	HPLC-UV	XBridge Phenyl 4.6×250mmmm, 5μm	H_2O/MeOH/H_3PO_4	210nm	USP
LAB. 025	HPLC-UV	XBridge Phenyl	H_2O/MeOH/H_3PO_4	210nm	USP
LAB. 026	HPLC-UV	Bridge C18 4.6mm×250mm, 5μm	H_2O/MeOH/H_3PO_4	210nm	USP
LAB. 027	HPLC-UV	Inertsil-Phenyl 4.6mm×250mm, 5μm	H_2O/MeOH/H_3PO_4	210nm	USP
LAB. 028	HPLC-UV	Inertsil ods-4 C18 4.6×250mmmm, 5μm	phosphate buffer/ MeOH/ACN	215nm	Ch.P.
LAB. 029	HPLC-UV	Alltima C18 4.6×150mmmm, 5μm	phosphate buffer/ MeOH/ACN	215nm	Ch.P.
LAB. 030	HPLC-UV	Alltima C18 4.6×150mmmm, 5μm	phosphate buffer/ MeOH/ACN	215nm	Ch.P.
LAB. 031	HPLC-UV	SHISEIDO C18 4.6×150mmmm, 5μm	phosphate buffer/ MeOH/ACN	215nm	Ch.P.
LAB. 032	HPLC-UV	SHIMADZU VP-ODS 4.6×250mmmm, 5μm	phosphate buffer/ MeOH/ACN	215nm	Ch.P.
LAB. 033	HPLC-UV	Agilent HC-C18 4.6×250mmmm, 5μm	phosphate buffer/ MeOH/ACN	215nm	Ch.P.
LAB. 036	HPLC-UV	USP Column L11 4.6×250mmmm, 5μm	H_2O:CH_3OH:H_3PO_4	210nm	USP
LAB. 037	HPLC-UV	X-Terra phynyl 4.6×250mmmm, 5μm	H_2O:CH_3OH:H_3PO_4	210nm	USP
LAB. 038	HPLC-UV	USP Column L11 4.6×250mmmm, 5μm)	H_2O:CH_3OH:H_3PO_4	210nm	USP

"/ " denotes data/information was not provided by participants

TABLE VII: System Suitability and Reference Material Information

Lab Code	Theoretical plates		Resolution	Reference Materials	
	Hydrochloro-thiazide	Captopril		Hydrochloro-thiazide	Captopril
LAB. 001	9000	15000	19.18	Sigma Aldrich	Sigma Aldrich
LAB. 002	7400	640	6.8	Sigma Aldrich	USP
LAB. 003	13310	1932	/	USP	USP
LAB. 004	2078	1530	7.48	USP	USP
LAB. 005	2747	2069	2.22	USP	USP
LAB. 006	/	/	/	Sigma Aldrich	Sigma Aldrich
LAB. 007	6435	728	7.3	USP	USP
LAB. 008	14105	10625	11.9	USP	USP
LAB. 009	4361	1944	2.19	USP	Sigma Aldrich
LAB. 010	3288	/	4.48	USP	USP
LAB. 011	5000	11500	23.9	USP	USP
LAB. 012	4080	646	7.25	USP	USP
LAB. 013	5436	3235	5.3	Sigma Aldrich	Sigma Aldrich
LAB. 014	6021	20417	3.3	Sigma Aldrich	Sigma Aldrich
LAB. 017	7652	7127	5.02	USP	USP
LAB. 019	>2000	>2000	>3	USP	USP
LAB. 020	7700	7000	6.0	Sigma Aldrich	Sigma Aldrich
LAB. 021	7595	3200	5.7/8.4	USP	In house standard
LAB. 022	3578	2391	6.36	BP	BP
LAB. 023	7412	1574	10.7	Indonesian Ph. RS	Indonesian Ph. RS

TABLE VII: System Suitability and Reference Material Information (Cont'd)

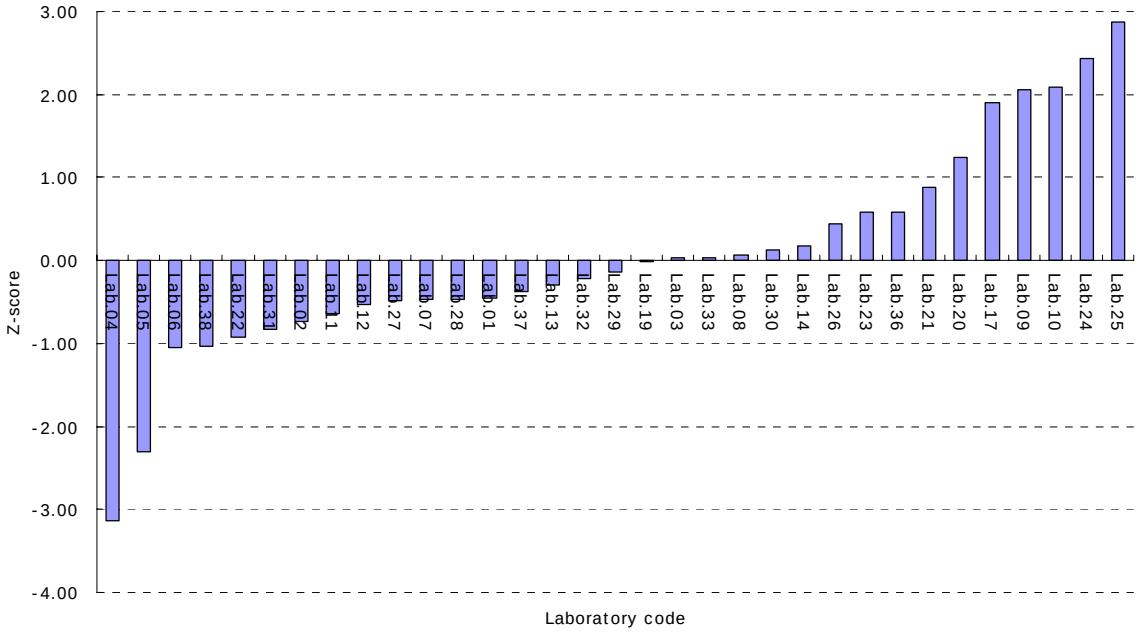
Lab Code	Theoretical plates		Resolution	Reference Materials	
	Hydrochloro-thiazide	Captopril		Hydrochloro-thiazide	Captopril
LAB. 024	6218	1068	9.9	Indonesian Ph. RS	Indonesian Ph. RS
LAB. 025	49174	7303	10.1	PPOMN	PPOMN
LAB. 026	23506	5795	4.3	Indonesian Ph. RS	Indonesian Ph. RS
LAB. 027	/	/	9.4	USP	USP
LAB. 028	10886	3493	13.8	NIFDC RS*	NIFDC RS
LAB. 029	8764	2575	6.4	NIFDC RS	NIFDC RS
LAB. 030	3700	900	7.3	NIFDC RS	NIFDC RS
LAB. 031	3551	1256	7.86	NIFDC RS	NIFDC RS
LAB. 032	13997	4440	17.4	NIFDC RS	NIFDC RS
LAB. 033	14556	2386	13.1	NIFDC RS	NIFDC RS
LAB. 036	9715	4895	3.3	Ranbaxy Laboratorie Ltd	USP
LAB. 037	4255	741	2	/	/
LAB. 038	11939	2207	10.5	USP	USP

"/ " denotes data/information was not provided by participants

"** NIFDC: National Institutes for Food and Drug Control

FIGURE I

(a)z-Scores of Participants' for Hydrochlorothiazide



(b) Mean concentration in % of Hydrochlorothiazide (in ascending order)

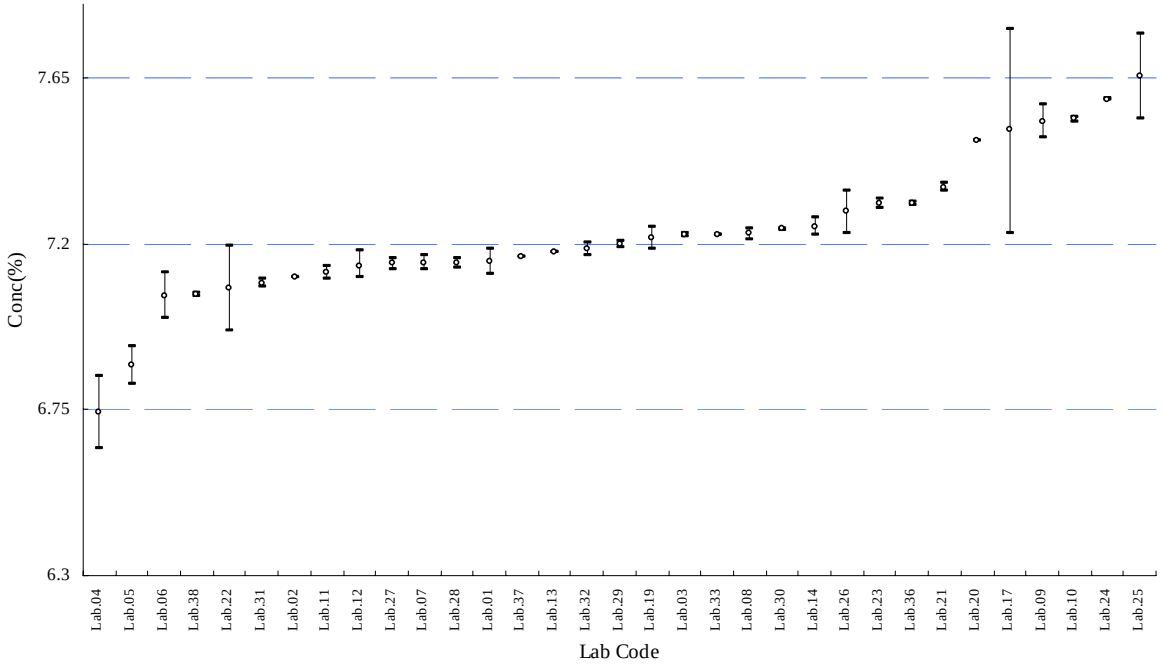
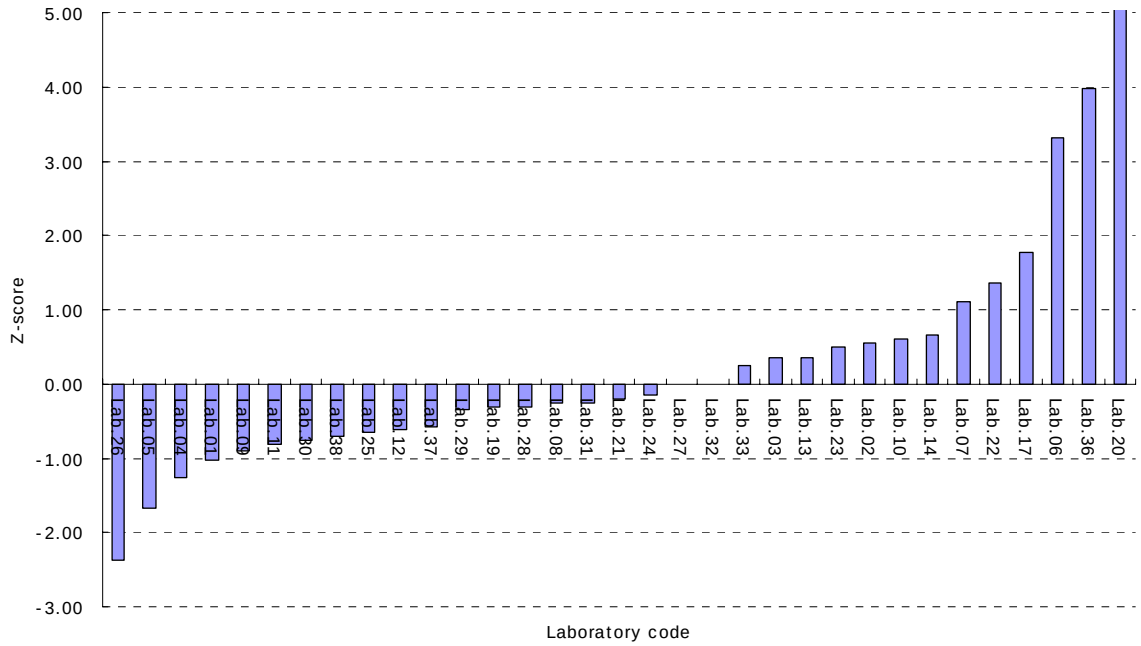
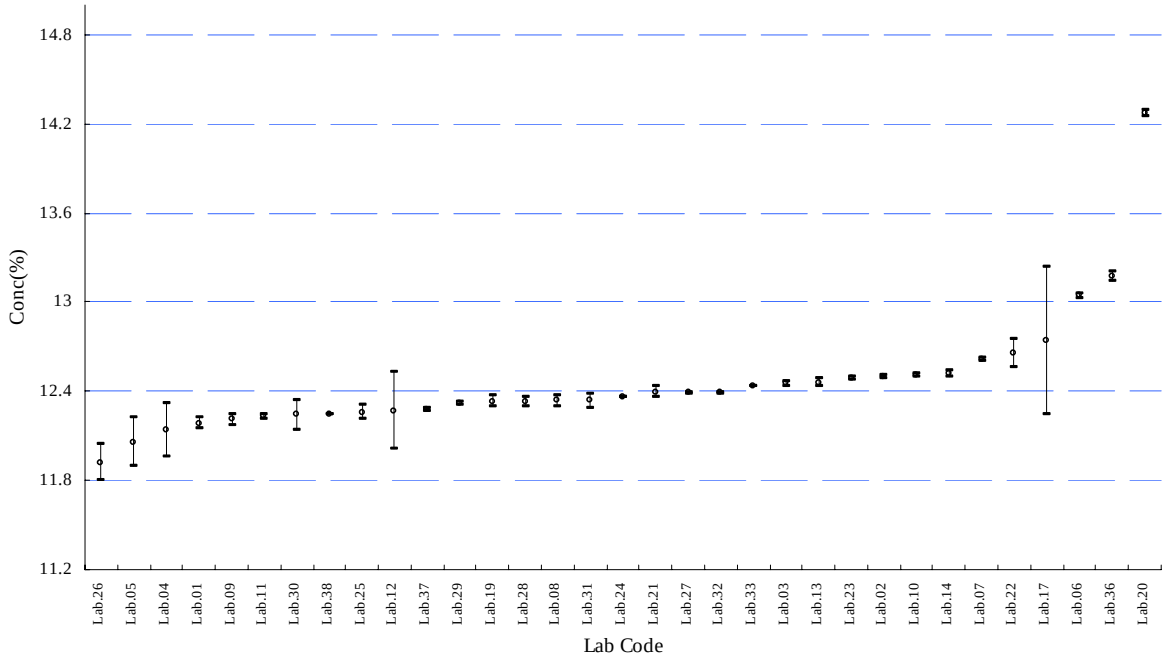


FIGURE II

(a)z-Scores of Participants' for Captopril



(b) Mean concentration in % of Captopril (in ascending order)



APPENDIX I : Preparation of Testing Material

Preparation of the test material was performed in accordance with ISO/IEC17043:2010. In brief, Captopril, Hydrochlorothiazide and suitable excipients were collected from a pharmaceutical company in China. The materials were ground to fine powder separately and then thoroughly mixed in high speed blender. Homogenized powder was then packed into brown glass bottles(2 g per bottle). More than 340 bottles of samples were finally prepared and stored at a temperature of below 30 ℃ before shipment.

APPENDIX II: Homogeneity Test

a. A total of 12 bottles of test material were randomly selected from the bulk. Two portions (sub-samples) of about 120mg each were taken from each bottle for duplicate analysis for Hydrochlorothiazide and Captopril in May 2011 using HPLC-UV.

b. Analysis was performed by the method of Assay of Captopril and Hydrochlorothiazide Tablets in USP using HPLC with the following conditions:

Model: Agilent 1100 HPLC with G1315B DAD detector

Injection volume: 20 μL

Mobil phase flow rate: 1.5 mL/min

Column: Zorbax Eclipse Plus Phenyl-Hexyl Analytical 4.6×250mm 5-Micron

Sample temperature: 5℃

Column temperature: 40℃

Detector wave length: 210nm

c. Results of the 24 sub-samples were as follows:

Sample Code	Concentration (%) of duplicate analysis			
	Hydrochlorothiazide		Captopril	
	#1	#2	#1	#2
6	7.283	7.265	12.50	12.63
16	7.281	7.288	12.59	12.57
35	7.309	7.256	12.67	12.59
48	7.319	7.289	12.71	12.50
84	7.273	7.276	12.67	12.65
130	7.321	7.285	12.71	12.65
139	7.312	7.285	12.66	12.67
203	7.380	7.320	12.80	12.62
294	7.306	7.283	12.60	12.69
301	7.302	7.328	12.62	12.82
306	7.338	7.304	12.66	12.54
326	7.249	7.281	12.54	12.63
Mean($\bar{x}$)	7.297		12.64	
SD(S_x)	0.029		0.079	
RSD(%)	0.39		0.63	

d. According to ISO13528:2005, the test materials are of adequate homogeneity if:

$S_s \leq 0.3\,\sigma_R$

where S_s = Between-samples standard deviation

σ_R = robust standard deviation

(Standard deviation for proficiency assessment)

S_s is calculated as:

$\sqrt{S_x^{\,2} - (S_w^{\,2}/2)}$

Where S_x = Standard deviation of sample averages

S_w = Within-samples standard deviation

S_w is calculated as:

$\sqrt{\sum W_t^{\,2}/(2n)}$

Where W_t = Absolute difference of the duplicate sample

n = Number tested samples

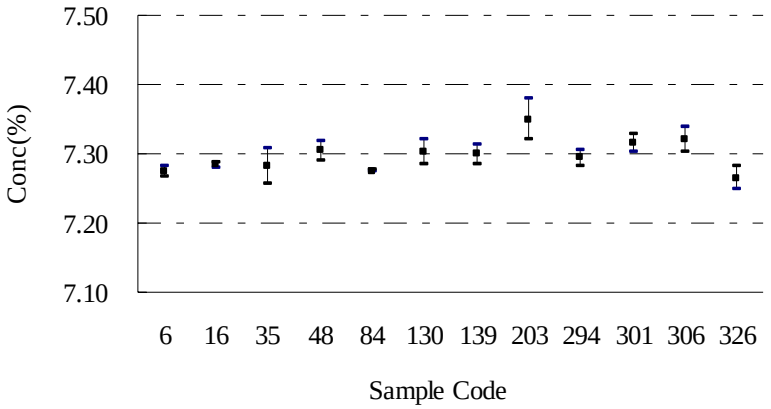
The results are summarized as follows:

Component	n	$\sum W_t^{\,2}$	S_w	S_x	S_s	**0.3** σ_R
Hydrochloro-thiazide	12	0.0131	0.0256	0.0287	0.0223	0.0456
Captopril	12	0.175	0.0935	0.0794	0.0439	0.0594

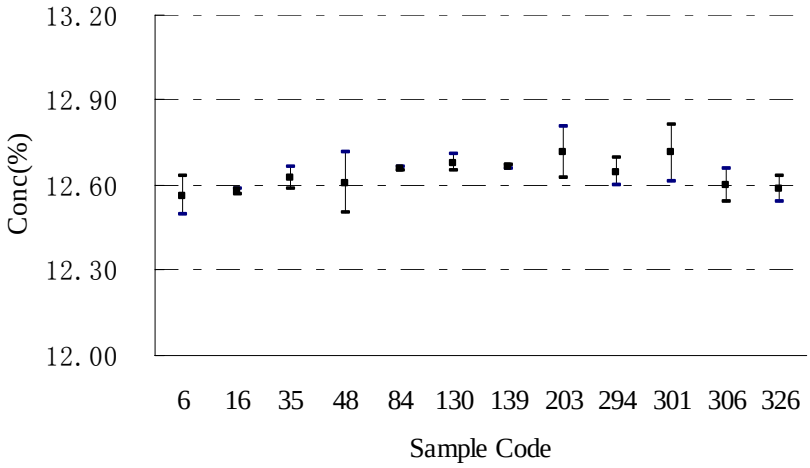
Since the between-samples standard deviations (S_s) from the homogeneity test for Hydrochlorothiazide and Captopril are less than the corresponding $0.3\,\sigma_R$, the homogeneity of the test materials are satisfactory.

e. Graphical illustrations of random samples in the homogeneity test

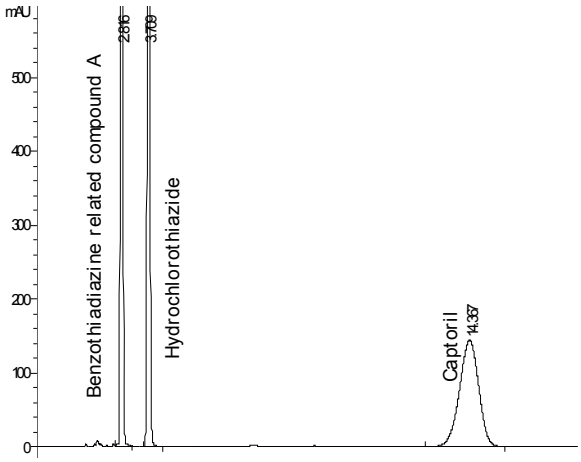
(i) Hydrochlorothiazide



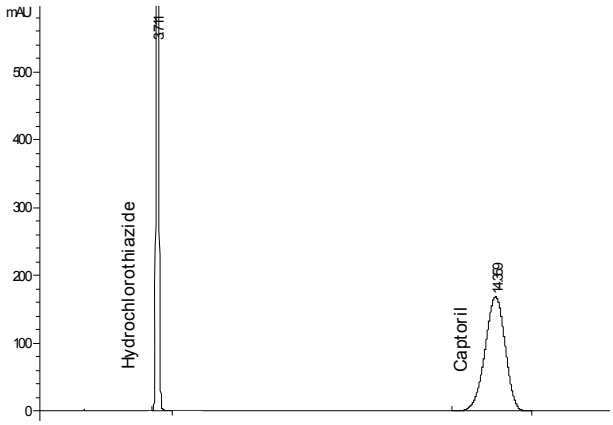
(ii) Captopril



f. Chromatogram of system suitability in the homogeneity test



g. A typical chromatogram for Hydrochlorothiazide and Captopril in the homogeneity test



APPENDIX III: Stability Test

a. Stability of Hydrochlorothiazide and Captopril in the test material was monitored in June 2011 and October 2011, which has fully covered the period from sample dispatch to result submission.

b. Samples selected for the stability test were stored at (i) a room temperature of 25℃and (ii) an elevated temperature of 40 ℃ Rh75%

c. Three samples from each temperature set was randomly selected and analyzed in duplicate under the same operational conditions as those in homogeneity test.

d. Stability of samples was determined using the absolute difference between the mean value obtained in homogeneity test ($\bar{x}$) and the mean value ($\overline{y_i}$) of each stability test. As stated in ISO13528:2005, the absolute difference should not be more than 30% of the standard deviation of the proficiency test (σ_R).

$$\text{ie. } \left|\bar{x} - \overline{y_i}\right| \leq 0.3\,\sigma_R$$

e. The results of stability tests are summarized below:

(i)at 25℃

period Sample code	Component	Concentration(%)					$\left\|\bar{x} - \overline{y_i}\right\|$	$0.3\,\sigma_R$	Status
		#1	#2	#3	$\overline{y_i}$	$\bar{x}$			
Jun 2011	Hydrochloro－thiazide	7.287	7.267	7.317	7.290	7.297	0.007	0.0456	Pass
	Captopril	12.60	12.59	12.68	12.63	12.64	0.01	0.0594	Pass
Jul 2011	Hydrochloro－thiazide	7.292	7.310	7.306	7.303	7.297	0.006	0.0456	Pass
	Captopril	12.67	12.56	12.56	12.60	12.64	0.04	0.0594	Pass
Oct 2011	Hydrochloro－thiazide	7.298	7.252	7.226	7.259	7.297	0.038	0.0456	Pass
	Captopril	12.64	12.68	12.54	12.62	12.64	0.02	0.0594	Pass

(ii)at 40℃

period Sample code	Component	Concentration(%)					$\lvert\bar{x}-\bar{y_i}\rvert$	$0.3\sigma_R$	Status
		#1	#2	#3	$\bar{y_i}$	$\bar{x}$			
Jun 2011	Hydrochloro—thiazide	7.267	7.288	7.333	7.296	7.297	0.001	0.0456	Pass
	Captopril	12.59	12.57	12.63	12.60	12.64	0.04	0.0594	Pass
Jul 2011	Hydrochloro—thiazide	7.320	7.283	7.300	7.301	7.297	0.004	0.0456	Pass
	Captopril	12.57	12.65	12.62	12.61	12.64	0.03	0.0594	Pass
Oct 2011	Hydrochloro—thiazide	7.276	7.298	7.333	7.302	7.297	0.005	0.0456	Pass
	Captopril	12.63	12.67	12.68	12.66	12.64	0.02	0.0594	Pass

f. The stability of Hydrochlorothiazide and Captopril inside the sample bottles is found to be satisfactory and the materials are suitable to be used in the PT program.

APPENDIX IV: T-test of the LOD

a. LOD of the test material was monitored in June 2011 and October 2011, which has fully covered the period from sample dispatch to result submission.

b. Samples selected for the test were stored at a temperature of 40 ℃ Rh75%

c. Results of the LOD of 12 samples in June 2011 were as follows:

Sample Code	LOD (%)
6	0.42
16	0.52
35	0.47
48	0.54
84	0.46
130	0.31
139	0.49
203	0.52
294	0.39
301	0.44
306	0.50
326	0.41
$\overline{x_1}$	0.46
SD(s_1)	0.066

d. There was no significent difference in the period if:

$t < t_{crit}$

where t = t-test statistics

t_{crit} = Critical value of the t-test

t-test statistics is calculated as:

$$t = \frac{\left|\overline{x_2} - \overline{x_1}\right|}{\sqrt{\frac{(n_1 - 1){s_1}^2 + (n_2 - 1){s_2}^2}{n_1 + n_2 - 2} \cdot \frac{n_1 + n_2}{n_1 \cdot n_2}}}$$

where n_1=12, n_2=3

e. Results of the LOD of 3 samples in Oct 2011 were as follows. Three samples were randomly selected and analyzed in duplicate under the same operational conditions.

period	**LOD(%)**					t	t_{crit}	**Status**
Sample code	**#1**	**#2**	**#3**	$\overline{x_2}$	s_2			
Oct 2011	0.36	0.39	0.48	0.41	0.059	1.085	2.160	Pass

f. The results of the t-test indicated that there was no significant difference in the period from June 2011 to November 2011. It means the sealed condition of the samples was fine.

APPENDIX V: Instructions to Accreditation Bodies

INSTRUCTIONS
(FOR ACCREDITATION BODIES)

1. SAMPLE

Accreditation bodies (AB) should receive the PT samples and instruction information (hard copy and soft copy) from the PT organizers. The samples consist of the sealed brown glass bottles that each contains about 2g of white powder，labeled as“APLAC T080 XXX”.

Upon receipt, AB should carefully check the samples (number of bottles, physical conditions, etc.), and shall promptly acknowledge **SIFDC** (Shanghai Institute for Food and Drug Control) by returning the “Receipt Form (for Accreditation Bodies)” electronically to **aplac_t080@sina.com**. New sample(s) will be replaced for any missing and damaged bottles.

AB are recommended to promptly distribute the samples to their nominated laboratories. Each participant will be given ONE bottle of sample. The samples shall be stored in a secure environment below 30°C before dispatch.

AB shall remind their nominated laboratories carefully check the sample upon receipt and promptly acknowledge **SIFDC** by returning the “Receipt Form (for Participants)” electronically to **aplac_t080@sina.com.**

2. RESULTS

AB shall inform their respective nominated laboratories to complete the Results Proforma (both part Ⅰ&Ⅱ) and submit electronically to the organizers on or before **10 September 2011** to **aplac_t080@sina.com**. (Notes: 1. Results submitted after the deadline will not be accepted by the organizers. 2. To avoid possible poor transmission quality, results returned by fax are generally not recommended).

3. ENQUIRIES

Please contact the organizers at **aplac_t080@sina.com** for further enquiries concerning the program**.**

APPENDIX VI: Receipt Form for Accreditation Bodies

RECEIPT FORM
(FOR ACCREDITATION BODIES)

From： _____

_____

_____

(Name of Accreditation Bodies)

_____

(Contact Person)

Email： _____

Date： _____

To: Ms. Yang Meicheng
Shanghai Institute for
Food and Drug Control

Email:aplac_t080@sina.com

I hereby acknowledge the receipt of sample(s) for the APLAC T080 program.

The sample is found (damaged / satisfactory)* and should be (suitable / unsuitable)* for laboratory testing.

Remark(s) ： _____

_____

_____

_____

Please circle as appropriate.

APPENDIX VII: Instruction to Participating Laboratories

INSTRUCTIONS (FOR PARTICIPANTS)

1. SAMPLE

(a) Each participant will be given ONE brown glass bottle, labeled as "APLAC T080 XXX" which contains about 2 g of white powder(equivalent to powdered tablets), from the organizers or via their respective accreditation bodies (AB).

(b) Upon receipt of the sample, participants should carefully inspect the sample for any physical damages and defects.

(c) Participants shall promptly acknowledge receipt of the sample by returning the "Receipt Form (for Participants)" electronically to SIFDC at **aplac_t080@sina.com**. New sample(s) will be replaced for any damaged claims.

(d) Intact sample is recommended to be stored in a secure environment at a temperature of below 30°C before use. Once the sample bottle is opened, the test must be performed immediately.

2. ANALYSIS

(a) Participants are required to determine the content of Captopril and Hydrochlorothiazide in the sample. The range of content is about 6%～20% for captopril and 5%～10% for hydrochlorothiazide.

(b) Participants should use suitable HPLC methods (could be pharmacopoeia and in-house methods, etc.), and record the method used on the Results Proforma.

(c) Participants should determine the loss on drying (Dry about 1 g sample in vacuum at 60℃ for 3 hours) , and record the results on the Results Proforma. This is for the organizors to monitor the sealed condition of sample bottles.

3. RESULTS

(a) Report all the data and the arithmetic mean in Part I of the "Results Proforma".

(b) Fill in the information of the analytical method you performed in Part II of the "Results Proforma".

(c) Submit the results electronically to the organizers on or before **10th September 2011**

at **aplac_t080@sina.com** using the soft copy of the "Results Proforma" provided. (Notes: 1. Results submitted after the deadline will not be accepted by the organizers. 2. To avoid possible poor transmission quality, results by fax are generally not recommended).

4. ENQURIES

Please contact the organizers at **aplac_t080@sina.com** for further enquiries concerning the program.

APPENDIX VIII: Receipt Form for Participating Laboratories

RECEIPT FORM
(FORPARTICIPANTS)

From： _____

_____

_____

(Name of Participant)

_____

(Contact Person)

_____

(Country/Economy)

To: Ms. Yang Meicheng
Shanghai Institute for
Food and Drug Control

Email： _____ **Email:aplac_t080@sina.com**

Date： _____

I hereby acknowledge the receipt of ONE sample for the APLAC T080 program.

The sample is found (damaged / satisfactory)* and should be (suitable / unsuitable)* for laboratory testing.

Remark(s) ： _____

_____

_____

*** Please circle as appropriate.**

APPENDIX IX: Results Proforma

RESULTS PROFORMA

<Both Part I & II **MUST** be completed by Participant >

Laboratory Code: _____

Sample Code: _____

Name of Participant : _____

Contact Person: _____

Email: _____

Signature: _____

Part I: Analytical Results

Ingredients	Content (%, based on as-received weight)		
	Data 1	Data 2	Mean
Captopril			
Hydrochlorothiazide			

Loss on drying (%) : _____

Note:

1. Report the values of contents to 4 significant figures;
2. Please submit this "Results Proforma" to the organizers by email to **aplac_t080@sina.com** before **10th September 2011.**

To be continued …

Part II: Method Information

Is the method used for the results submitted in your scope of accreditation?		☐ yes		☐ no	
Reference	Captopril	☐ standard ☐ journal ☐ book ----------	year	No./Vol /Edition	pages
	Hydrochlorothiazide	☐ standard ☐ journal ☐ book ----------	year	No./Vol /Edition	pages
Reference Materials	Captopril	Supplier(s): Purity:			
	Hydrochlorothiazide	Supplier(s): Purity:			
Chromatographic system	Instrument model				
	HPLC column	*eg.Symmetry, C18 (150 mm×2.1 mm, 5 μm)*			
	Mobile phase				
	Detective wavelength				
System suitability	Theoretical plates	Captopril: Hydrochlorothiazide:			
	RSD of replicate injection	Captopril: Hydrochlorothiazide:			
	Resolution	(please specify)			
Standard preparation	(please specify)				
Sample preparation	(please specify)				
Spectrogram	Spectrograms Attached: _____Pages				
Additional information					