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Australian Government
Department of Health
Therapeutic Goods Administration

Office of Laboratories and Scientific Services

Operations: HPLC Manual	
Procedure	HPLC – 01 – General HPLC – WORKSHEET
Written	s22 [redacted]
Authorised	s22 [redacted]
Date issued	27/6/2014
Revision #	6

HPLC – 01 – General HPLC – WORKSHEET

TEST DETAILS			
TEST NAME	Size-exclusion chromatography ✓		
METHOD REFERENCE	HPLC-10-IgG SE-HPLC - SOP ✓		
METHOD MODIFICATIONS (if any)	N/A		
MODIFICATIONS APPROVED BY:			
NAME OF ANALYST	s22 [redacted]	TEST DATE	07/02/2017 ✓

BUFFERS AND SOLUTIONS	
SOLUTIONS	**BATCH No:**
MOBILE PHASE **A**	MB07Feb2017-1 ✓
MOBILE PHASE **B**	N/A
SAMPLE DILUENT	Sodium chloride solution 0.9% MB07Feb2017-2 ✓

PIPETTES USED AND EXPIRY DATES	
5643 200-1000µL Exp: 27 Apr 2017	32524 1mL-10mL Exp: 21 Apr 2017
32678 20-200µL Exp: 07/05/2017	
5648 40-200µL Exp: 16/Apr/2017	

REFERENCE MATERIALS	
NAME AND CODE	BATCH NO:
EDQM, European Pharmacopeia Commission - Human	
Immunoglobulin	BRP Batch 1.2

REFERENCE MATERIAL PREPARATIONS AND CALCULATIONS

Reconstituted the whole content vial (Human Immunoglobulin), with 7.0mL of ultra pure H_2O. leave at room temp for at least 2 hours. The finial concentration is 100 mg/mL IgG.

From 100mg/mL to further dilute to 40mg/mL:

200µL of Ref Std [100mg/mL] + 300µL of 0.9% sodium chloride solution = 40mg/mL

Injection volume = 10µL. ✓

For stability study: Using old Ref. Std that aliquot on 02/09/2014 with concentration 100mg/mL. (ULF1 - 1A)

Human Ig (molecular si.) BRP
Batch 1.2 alq: 02/09/ 1
~ 300 uL

Dilution:

200µL of Old Ref Std [100mg/mL] + 300µL of 0.9% sodium chloride solution = 40mg/mL

Injection volume = 10µL. ✓

SYSTEM SUITABILITY CRITERIA AND RESULTS

PARAMETERS	LIMITS	RESULTS	COMMENTS
System Suitability ⇒ The relative retention time of the dimer peak to the monomer peak of the reference preparation should be about	s47(1)(b)	mean @ start = 0.84 ✓ mean @ End = 0.84 ✓ mean all inj = 0.84 ✓	Pass Pass ✓ Pass
⇒ The % RSD of the peak area for the monomer peak of the ref. preparation from triplicate injection at the start and at the end, bracking sample, should be	s47(1)(b)	Start = 0.10 End = 0.12 ✓ All = 0.57 ✓	Pass Pass ✓ Pass ✓
Sample Suitability ⇒ The relative retention time of the dimer and monomer peak of the sample, relative to the corresponding peaks of the ref. preparation should be (For IgG only)	s47(1)(b)	1610003595 M = 1.00 D = 1.00 ✓ 1610003596 M = 1.00 D = 1.00 ✓ 1610003597 M = 1.00 D = 1.00 (0.9918 ASC 16 2 17) 1610003610 M = 1.00 D = 1.00 (0.9992 16 2 17 ASC) 1610003684 M = 1.00 D = 1.00 ✓ 1610003685 M = 1.00 D = 1.00 ✓ 1610003682 M = 0.999 D = 1.00 ✓ 1610003683 M = 0.999 D = 1.00 ✓	} Pass
⇒ The % RSD of the peak area for the monomer peak of the sample from triplicate injections should be (for both IgG & albumin)	s47(1)(b)	1610003595 = 0.5 ✓ 1610003596 = 0.1 ✓ 1610003597 = 0.3 ✓ 1610003610 = 0.7 ✓ 1610003684 = 0.5 ✓ 1610003685 = 0.7 ✓ 1610003682 = 0.6 ✓ 1610003683 = 0.8 ✓	} Pass

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SAMPLE DETAILS	CSL Normal Immunoglobulin VF		
SAMPLE NAME	Normal Immunoglobulin VF (Human) 800mg/5mL Injection vial		
LIMS No:	1610003685		
BATCH No:	3620050074	EXPIRY:	20/05/2017.

SAMPLE DILUTIONS, CALCULATIONS and DATA

Initial Conc.	Vol. sample	Vol. diluent	Final Conc.	DF	Inj. Vol.
(160 mg/mL) 800mg/5mL	200µL	600µL	40mg/mL	4	10µL

Ref Std : RT for monomer = 27.660 ✓
RT for dimer = 23.208 ✓

Sample : RT for monomer = 27.676 ✓
RT for dimer = 23.231 ✓

Ratio : monomer = $\frac{27.676}{27.660}$ = 1.00 ✓

dimer = $\frac{23.231}{23.208}$ = 1.00 ✓

DATA LOCATIONS

Hard copies of Empower reports attached?	YES / (NO)	Data location in TRIM	D17-101835

TEST RESULTS

PARAMETERS	LIMITS	RESULTS	COMMENTS
Aggregates	s47(1)(b) ✓	4.40 % ✓	Pass
IgG monomer + dimer	s47(1)(b) ✓	95.60 % ✓	Pass
Fragments	s47(1)(b) ✓	Not detected ✓	

SAMPLE RESULTS: ☑ PASS ☐ FAIL

Signature of analyst: s22 ..... Date: 08 / 02 / 2017

Checked by: ..... s22 ..... Date: 16 / 02 / 2017

Entered in LIMS by: s22 ..... Date: 09 / 02 / 2017.

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Operations	Biochemistry – Forms
Procedure	Forms – Reagent Preparation Record
Written	s22 [redacted]
Authorised	s22 [redacted]
Date issued	20/06/2014
Revision #	3

Reagent Name: IgG Mobile phase (SE) ÷ Final Volume: 2 L

Prepared by:	Date:	Expiry Date:	Batch #: eg. LB01OCT09-1	Storage Temperature:
s22 [redacted]	07/02/2017	21/02/2017	MB07Feb2017 – 1	Room Temp.

Component	Manufacturer	Batch #	Required Amount	Measured Amount	Balance / Pipette LIMS #	Final Conc (%, mM, etc)
Sodium phosphate dibasic dihydrate	SIGMA-Aldrich	SZB92520	9.746g	9.746g	32126	0.05M
Sodium phosphate monobasic monohydrate	s22 [redacted] SLBD7295V	Sigma-Aldrich	3.482g	3.483g	"	0.03M
Sodium chloride	Merck	K45393104412	23.376g	23.376g	"	0.4M
Ultra pure H_2O	—	—	2 L	2 L	vol. flask.	

pH Adjustment	Target pH:	Initial pH:	Adjusted with:	Final pH:	pH Electrode LIMS#: (circle)
	N/A	6.9 N/A s22 [redacted]	HCl / HNO_3 / H_2SO_4 / H_3PO_4 NaOH / KOH / Other: N/A	6.9 N/A s22 [redacted]	8165 / 32109 N/A

Filtration (circle): None 0.45µm (0.2µm) Other: _____

Other Comments:

Attach Balance P

001:N + 9.745 g
002:N + 3.423 g
003:N + 23.376 g
07-Feb-2017 08:15:25

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Office of Laboratories and Scientific Services

Operations	Biochemistry – Forms
Procedure	Forms – Reagent Preparation Record
Written	[redacted]
Authorised	[redacted]
Date issued	20/06/2014
Revision #	3

Reagent Name: Sodium Chloride Solution (0.9%)

Final Volume: 100 mL

Prepared by:	Date:	Expiry Date:	Batch #: eg. LB01OCT09-1	Storage Temperature:
[redacted]	07/02/2017	21/02/2017	MB07Feb2017-2 ✓	R.T.

Component	Manufacturer	Batch #	Required Amount	Measured Amount	Balance / Pipette LIMS #	Final Conc (%, mM, etc)
Sodium Chloride	Merck	K45393104412	0.9g	0.901g.	32126	0.9%
Ultra pure H_2O	—		q.s to 100ml	q.s to 100mL	vol. flask	

pH Adjustment	Target pH:	Initial pH:	Adjusted with:	Final pH:	pH Electrode LIMS#: (circle)
	N/A	N/A	HCl / HNO_3 / H_2SO_4 / H_3PO_4 NaOH / KOH / Other: N/A	N/A	8165 / 32109 N/A

Filtration (circle):	None	0.45µm	(0.2µm)	Other: ______

Other Comments:

Attach Balance F[...]

0045N - 0.901 g
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