Macropathology and histopathology - individual findings for animals killed after 13 weeks of treatment

Group	:	1	2	3	4
Compound	:	Control	------	ABM-FD	------
Dosage (mg/kg/day)	:	0	500	1000	2000

ANIMAL NUMBER: 0103 SEX: FEMALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 07-FEB-05 STUDY DAY OF DEATH: 96 STUDY WEEK OF DEATH: 14 TERMINAL BODY WEIGHT: 320.9 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
	HEART : -CARDIOMYOPATHY,-SLIGHT
	LIVER : -HEPATOCYTE VACUOLATION, PERIPORTAL,-MINIMAL
LUNGS & BRONCHI : -PALE AREA(S); MULTIPLE PUNCTATE FOCI, ON LEFT LOBE AND RIGHT CAUDAL LOBE.	LUNGS & BRONCHI : >NO SIGNIFICANT LESION

Macropathology and histopathology - individual findings for animals killed after 13 weeks of treatment

Group	:	1	2	3	4
Compound	:	Control	------	ABM-FD	------
Dosage (mg/kg/day)	:	0	500	1000	2000

ANIMAL NUMBER: 0104 SEX: FEMALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 07-FEB-05 STUDY DAY OF DEATH: 96 STUDY WEEK OF DEATH: 14 TERMINAL BODY WEIGHT: 276.3 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

*** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

Macropathology and histopathology - individual findings for animals killed after 13 weeks of treatment

Group	:	1	2	3	4
Compound	:	Control	------	ABM-FD	------
Dosage (mg/kg/day)	:	0	500	1000	2000

ANIMAL NUMBER: 0105 SEX: FEMALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 07-FEB-05 STUDY DAY OF DEATH: 96 STUDY WEEK OF DEATH: 14 TERMINAL BODY WEIGHT: 325.7 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
	LN AXILLARY : -PARACORTEX - INCREASED CELLULARITY,-SLIGHT
	LN MESENTERIC : -PARACORTEX - INCREASED CELLULARITY,-SLIGHT
SKIN/SUBCUTIS : -SCAB(S); FEW DARK SCABS, <2X2MM, ON UPPER DORSAL THROAX.	SKIN/SUBCUTIS : -SCAB(S),-MODERATE -EPIDERMAL ULCERATION,-SLIGHT -EPIDERMAL HYPERPLASIA,-MODERATE
UTERUS : -FLUID DISTENTION; <7MM DIA.	UTERUS : -LUMINAL DILATATION,-MODERATE

Macropathology and histopathology - individual findings for animals killed after 13 weeks of treatment

Group	:	1	2	3	4
Compound	:	Control	------	ABM-FD	------
Dosage (mg/kg/day)	:	0	500	1000	2000

Protocol Number: IKI 001

ANIMAL NUMBER: 0106 SEX: FEMALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 07-FEB-05 STUDY DAY OF DEATH: 96 STUDY WEEK OF DEATH: 14 TERMINAL BODY WEIGHT: 322.5 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
	LIVER : -INFLAMMATION, PORTAL,-MINIMAL
LN MANDIBULAR : -ENLARGED; <10X8X4MM.	LN MANDIBULAR : -PARACORTEX - INCREASED CELLULARITY,-SLIGHT -PLASMACYTOSIS,-SLIGHT
SKIN/SUBCUTIS : -THICKENED; ON DORSAL SURFACE OF MUZZLE.	SKIN/SUBCUTIS : -EPIDERMAL HYPERPLASIA,-SLIGHT -DERMAL INFLAMMATION,-SLIGHT
	URINARY BLADDER : >TISSUE MISSING

Macropathology and histopathology - individual findings for animals killed after 13 weeks of treatment

Group :	1	2	3	4
Compound :	Control	------	ABM-FD	------
Dosage (mg/kg/day) :	0	500	1000	2000

ANIMAL NUMBER: 0107 SEX: FEMALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 07-FEB-05 STUDY DAY OF DEATH: 96 STUDY WEEK OF DEATH: 14 TERMINAL BODY WEIGHT: 306.5 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
UTERUS :	UTERUS :
-FLUID DISTENTION; <4MM DIA.	-LUMINAL DILATATION,-SLIGHT

Macropathology and histopathology - individual findings for animals killed after 13 weeks of treatment

Group	:	1	2	3	4
Compound	:	Control	------	ABM-FD	------
Dosage (mg/kg/day)	:	0	500	1000	2000

ANIMAL NUMBER: 0108 SEX: FEMALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 07-FEB-05 STUDY DAY OF DEATH: 96 STUDY WEEK OF DEATH: 14 TERMINAL BODY WEIGHT: 314.9 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
LN AXILLARY : -REGIONAL TO MASS	LN AXILLARY : >NO SIGNIFICANT LESION
PAWS : -MASS 1, PRESENT; HARD, SUBCUTANEOUS MASS, 2X2X2MM, ON MEDIAL SURFACE OF RIGHT FOREPAW.	PAWS : -EXOSTOSES,-PRESENT >NOTE:>MASS 1 = EXOSTOSES.
STOMACH : -ANTRUM WHITE NODULE(S); 3 PUNCTATE AREAS.	STOMACH : -ECTOPIC NONGLANDULAR EPITHELIUM IN GLANDULAR REGION, FOCAL,-PRESENT

Macropathology and histopathology - individual findings for animals killed after 13 weeks of treatment

Group	:	1	2	3	4
Compound	:	Control	------	ABM-FD	------
Dosage (mg/kg/day)	:	0	500	1000	2000

ANIMAL NUMBER: 0111 SEX: FEMALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 08-FEB-05 STUDY DAY OF DEATH: 97 STUDY WEEK OF DEATH: 14 TERMINAL BODY WEIGHT: 288.9 GRAMS

PATHOLOGY OBSERVATIONS

NECROPSY	HISTOPATHOLOGY
	LUNGS & BRONCHI : -ALVEOLAR HAEMORRHAGE,-SLIGHT
THYMUS : -DARK AREA(S); FEW PUNCTATE FOCI.	THYMUS : >NO SIGNIFICANT LESION

Macropathology and histopathology - individual findings for animals killed after 13 weeks of treatment

Group	:	1	2	3	4
Compound	:	Control	------	ABM-FD	------
Dosage (mg/kg/day)	:	0	500	1000	2000

ANIMAL NUMBER: 0112 SEX: FEMALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 08-FEB-05 STUDY DAY OF DEATH: 97 STUDY WEEK OF DEATH: 14 TERMINAL BODY WEIGHT: 313.0 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
KIDNEYS : -DARK AREA(S); MULTIPLE PUNCTATE FOCI.	KIDNEYS : -CORTICAL TUBULAR BASOPHILIA,-MODERATE -TUBULAR HAEMORRHAGE,-SLIGHT
LN MANDIBULAR : -ENLARGED; <15X6X3MM.	LN MANDIBULAR : -PARACORTEX - INCREASED CELLULARITY,-SLIGHT -PLASMACYTOSIS,-MODERATE
LUNGS & BRONCHI : -CONGESTED; WT 2.039G.	LUNGS & BRONCHI : -ALVEOLAR HAEMORRHAGE,-MODERATE
	THYMUS : -CORTEX - REDUCED CELLULARITY,-MINIMAL

Macropathology and histopathology - individual findings for animals killed after 13 weeks of treatment

Group	:	1	2	3	4
Compound	:	Control	------	ABM-FD	------
Dosage (mg/kg/day)	:	0	500	1000	2000

ANIMAL NUMBER: 0113 SEX: FEMALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 08-FEB-05 STUDY DAY OF DEATH: 97 STUDY WEEK OF DEATH: 14 TERMINAL BODY WEIGHT: 310.3 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
DUODENUM : -PALE AREA(S); PUNCTATE FOCUS.	DUODENUM : >NO SIGNIFICANT LESION
SKIN/SUBCUTIS : -TRAUMA; LEFT PINNA PARTIALLY ABSENT.	

Macropathology and histopathology - individual findings for animals killed after 13 weeks of treatment

Group	:	1	2	3	4
Compound	:	Control	------	ABM-FD	------
Dosage (mg/kg/day)	:	0	500	1000	2000

ANIMAL NUMBER: 0114 SEX: FEMALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 08-FEB-05 STUDY DAY OF DEATH: 97 STUDY WEEK OF DEATH: 14 TERMINAL BODY WEIGHT: 257.3 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
	KIDNEYS : -CORTICAL TUBULAR BASOPHILIA,-MINIMAL -MINERALISATION, MEDULLA,-MINIMAL
LUNGS & BRONCHI : -CONGESTED; 3 POORLY DEFINED AREAS, ON RIGHT CAUDAL, LEFT AND AZYGOUS LOBES.	LUNGS & BRONCHI : -ALVEOLAR HAEMORRHAGE,-MODERATE
	STOMACH : -DILATED GLANDS,-MINIMAL
UTERUS : -FLUID DISTENTION; <5MM DIA.	UTERUS : -LUMINAL DILATATION,-MODERATE

Macropathology and histopathology - individual findings for animals killed after 13 weeks of treatment

Group	:	1	2	3	4
Compound	:	Control	------	ABM-FD	------
Dosage (mg/kg/day)	:	0	500	1000	2000

Protocol Number: IKI 001

ANIMAL NUMBER: 0115 SEX: FEMALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 08-FEB-05 STUDY DAY OF DEATH: 97 STUDY WEEK OF DEATH: 14 TERMINAL BODY WEIGHT: 293.4 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
	KIDNEYS : -MINERALISATION, MEDULLA,-MINIMAL

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

Macropathology and histopathology - individual findings for animals killed after 13 weeks of treatment

Group	:	1	2	3	4
Compound	:	Control	------	ABM-FD	------
Dosage (mg/kg/day)	:	0	500	1000	2000

ANIMAL NUMBER: 0116 SEX: FEMALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 08-FEB-05 STUDY DAY OF DEATH: 97 STUDY WEEK OF DEATH: 14 TERMINAL BODY WEIGHT: 333.4 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
	KIDNEYS : -MINERALISATION, CORTEX,-MINIMAL
	THYROIDS : -C-CELL HYPERPLASIA,-MINIMAL
UTERUS : -FLUID DISTENTION; <6MM DIA.	UTERUS : -LUMINAL DILATATION,-SLIGHT

Macropathology and histopathology - individual findings for animals killed after 13 weeks of treatment

Group	:	1	2	3	4
Compound	:	Control	------	ABM-FD	------
Dosage (mg/kg/day)	:	0	500	1000	2000

ANIMAL NUMBER: 0117 SEX: FEMALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 08-FEB-05 STUDY DAY OF DEATH: 97 STUDY WEEK OF DEATH: 14 TERMINAL BODY WEIGHT: 342.0 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
	LIVER : -CLEAR CELL FOCI,-PRESENT LN AXILLARY : -DILATED/CYSTIC SINUSES,-SLIGHT LN MESENTERIC : -PARACORTEX - INCREASED CELLULARITY,-SLIGHT

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

Macropathology and histopathology - individual findings for animals killed after 13 weeks of treatment

Group	:	1	2	3	4
Compound	:	Control	------	ABM-FD	------
Dosage (mg/kg/day)	:	0	500	1000	2000

ANIMAL NUMBER: 0118 SEX: FEMALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 08-FEB-05 STUDY DAY OF DEATH: 97 STUDY WEEK OF DEATH: 14 TERMINAL BODY WEIGHT: 356.8 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
	BRAIN : -DILATED VENTRICLES,-PRESENT
LN MANDIBULAR : -ENLARGED; <11X6X3MM.	LN MANDIBULAR : -PARACORTEX - INCREASED CELLULARITY,-SLIGHT -PLASMACYTOSIS,-SLIGHT -INCREASED GERMINAL CENTRE DEVELOPMENT,-SLIGHT
	LUNGS & BRONCHI : -ALVEOLAR HAEMORRHAGE,-SLIGHT

Macropathology and histopathology - individual findings for animals killed after 13 weeks of treatment

Group	:	1	2	3	4
Compound	:	Control	------	ABM-FD	------
Dosage (mg/kg/day)	:	0	500	1000	2000

ANIMAL NUMBER: 0119 SEX: FEMALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 08-FEB-05 STUDY DAY OF DEATH: 97 STUDY WEEK OF DEATH: 14 TERMINAL BODY WEIGHT: 294.1 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
	PARATHYROIDS :
	>TISSUE MISSING

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

Macropathology and histopathology - individual findings for animals killed after 13 weeks of treatment

Group :	1	2	3	4
Compound :	Control	------ ABM-FD ------		
Dosage (mg/kg/day) :	0	500	1000	2000

ANIMAL NUMBER: 0120 SEX: FEMALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 08-FEB-05 STUDY DAY OF DEATH: 97 STUDY WEEK OF DEATH: 14 TERMINAL BODY WEIGHT: 270.1 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
	LN MESENTERIC : -SINUS HISTIOCYTOSIS,-SLIGHT
UTERUS : -FLUID DISTENTION; <5MM DIA.	UTERUS : -LUMINAL DILATATION,-SLIGHT

ANNEX 1

Formulation chemistry report

ABM-FD

FORMULATION CHEMISTRY

Authors:

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INTRODUCTION

This report describes the analytical and sampling procedures used and details the results obtained for:

- The validation of the analytical procedure (HRC/FCH/M80/04 issue 01/011104)[Ref.2] established at Huntingdon Life Sciences for the determination of the glucose content of ABM-FD in aqueous formulations. The analytical procedure was based on methodology supplied by the Sponsor[Ref.1].

  The analytical method involved enzyme and acid hydrolysis of the test sample and determination of the resulting glucose content by CLS personnel at the Huntingdon Research Centre.

- The glucose content of ABM-FD in aqueous formulations during refrigerated and ambient temperature storage at nominal concentrations of 50 mg/mL and 200 mg/mL.

- The glucose content of ABM-FD in aqueous formulations in test formulations analysed during the study.

The formulations for this study were prepared as solutions of ABM-FD in water by Formulation Chemistry and Pharmacy personnel. The analytical work was undertaken by Formulation Chemistry and Central Laboratory Services (CLS) personnel between 20 October 2004 and 01 February 2005.

EXPERIMENTAL PROCEDURE

ANALYTICAL PROCEDURE

Test substance

Identity:	ABM-FD
Supplier:	Iwade Research Institute of Mycology.
Batch number:	EX 200408.

Apparatus and instrumentation

Spectrophotometer:	Hitachi 917 Analyzer, Roche Diagnostics.
Wavelength:	340 – 700 nm.

pH Meter.

Balances fitted with printers:	Capability of weighing to accuracies of 0.1 mg.

General laboratory apparatus and glassware.

Reagents

Control vehicle:	Water for formulation.
Sodium dihydrogen orthophosphate dihydrate:	Certified grade.
Di-sodium hydrogen orthophosphate dihydrate:	Certified grade.
Orthophosphoric acid:	Certified grade.
Sodium hydroxide:	Certified grade.
Hydrochloric acid S.G. 1.16:	Certified grade.
Sulphuric Acid S.G. 1.84:	Certified grade.
Acetone:	Certified grade.
Ethanol:	Certified grade.
Water:	Deionised reverse osmosis.

Reagents (continued)

α-Amylase, heat stable.

Protease from *Bacillus lichenformis*.

Amyloglucosidase from *Aspergillus* niger, solution.

Celite, acid-washed.

Sample process

Enzyme Hydrolysis: A representative sample (5 mL) of each formulation was accurately dispensed into a glass beaker. Suitable volumes of phosphate buffer, pH 6.0 (20 mL) and α-amylase (0.1 mL) were added and the beaker was covered with aluminium foil and placed in a water bath for 30 minutes, with gentle agitation every 5 minutes. The extract was cooled to ambient temperature and freshly prepared protease solution (50 mg/mL, 0.1 mL) was added. The beaker was placed again in a water bath (60°C) for 30 minutes with gentle agitation every 5 minutes.

The extract was cooled to ambient temperature and the pH of each solution adjusted to 4.3 using hydrochloric acid (0.375M). Amyloglucosidase (0.1 mL) was added and the beaker placed in a water bath (60°C) for 30 minutes with gentle agitation every 5 minutes. The extract was transferred to a volumetric flask and diluted to volume using ethanol. The flask was stored at ambient temperature overnight to allow complete precipitation of the solids.

The extract was filtered and the residue washed with aqueous ethanol (80% v/v) and acetone.

Acid hydrolysis: The filter paper and contents were transferred into a glass bottle, sulphuric acid was added and the sealed bottle placed on a spiramix for 4 hours. The contents of the bottle were transferred using water into a beaker, covered with aluminium foil and stored at ambient temperature overnight.

The beaker was placed in boiling water for 2 hours. The extract was cooled and the pH adjusted to 7.0 using sodium hydroxide. The extract was filtered and diluted to volume. The above procedure was repeated at each occasion for a regent blank, which was used to correct the sample concentrations. The glucose content of the final solution was determined by CLS personnel using the Gluco-quant® Glucose /HK method. From the glucose content it was possible to determine the ß-glucan content and the concentration of ABM-FD using the following equations.

Calculation

Determine the concentration of ABM-FD using the following equation:

$$\text{Concentration of ABM - FD, mg/mL} = ((G \times \frac{MW}{1000} \times 0.9 \times \frac{100}{P} \times \frac{D}{V}) - B) \times \frac{100}{R}$$

Determine procedural recovery values using the following equation:

$$\text{Procedural recovery value, \%} = \frac{\text{Analysed concentration, mg/mL}}{\text{Fortified concentration, mg/mL}} \times 100$$

Where	G	=	Glucose concentration (mM)
	MW	=	Molecular weight of glucose (180)
	1000	=	Conversion factor for mM to M.
	V	=	Sample volume (5 mL)
	D	=	Dilution volume (mL)
	P	=	ß-glucan content of the test item (2.9%)
	B	=	Concentration calculated from Reagent Blank (mg/mL)
	0.9	=	Conversion factor for glucose to ß-glucan.
	R	=	Appropriate procedural recovery value at analysis

VALIDATION OF THE ANALYTICAL PROCEDURE

The analytical procedure was validated by determining a minimum of six procedural recoveries to cover the range of 50 mg/mL to 200 mg/mL.

Procedural recoveries were prepared by the addition of control matrix (water) to an appropriate number of vials containing ABM-FD. The prepared procedural recoveries were analysed in accordance with the analytical procedure.

GLUCOSE CONTENT OF ABM-FD IN AQUEOUS FORMULATIONS DURING STORAGE

The glucose content of ABM-FD in aqueous formulations was assessed at nominal concentrations of 50 mg/mL and 200 mg/mL, during ambient and refrigerated storage. Freshly prepared specimen formulations (Ca 120 mL) were equally sub-divided (4 × 30 mL) into four amber glass screw-top bottles by Chemistry personnel and analysed as detailed below.

Ambient temperature storage (nominally +21°C)

The contents of one bottle of each formulation were mixed by 20-fold inversion. After mixing, duplicate samples (5 mL) were removed for analysis from the middle of the formulation immediately (representing 0 hours) and after 4 hours and 24 hours ambient temperature storage.

Refrigerated storage (nominally +4°C)

The remaining bottles were refrigerated and on Days 1 and 8, the appropriate bottle was removed from storage and equilibrated to ambient temperature for 1 hour. The contents of the bottle were mixed by 20-fold inversion and duplicate samples (5 mL) were removed for analysis from the middle of the formulation.

CONCENTRATION IN TEST FORMULATIONS

At Weeks 1 and 12 of treatment, freshly prepared test formulations were sampled (25 mL) by Pharmacy personnel and submitted for analysis. On receipt, each treated sample was sub-sampled in duplicate (2 × 5 mL) and analysed in accordance with the analytical procedure.

REFERENCES

1. ß-glucan analytical method, client e-mail of 25 June 2004.

2. Fleet D., Johnson, S.F., Mann, P., (2004). Analysis of ABM-FD in aqueous formulations. Huntingdon Life Sciences Analytical Method HRC/FCH/M80/04 Issue 01/011104.

RESULTS AND DISCUSSION

Method validation

The analytical procedure was validated for ABM-FD in water with respect to the method accuracy and precision and the results are presented in Table 1: a mean procedural recovery value of 94.9% (CV=6.59%, n=10) was obtained for concentrations in the range 50 mg/mL to 200 mg/mL.

Formulation trials

The glucose content of ABM-FD in aqueous formulations was confirmed as consistent at nominal concentrations of 50 mg/mL and 200 mg/mL during storage at ambient temperature for 1 day and refrigerated storage for 8 days (Table 2). At each time-point, the mean analysed concentration for the duplicate samples remained within 20% of the initial time zero value. The slight day-to-day variation in the sample results is attributed to the complexity of the analytical method. Following extended storage, microbial growth was observed in the formulation and analysis of the Day 15 samples was not undertaken.

Concentrations in dose formulations

The mean concentrations of ABM-FD, analysed with respect to the glucose content, in test formulations and the deviation of mean results from nominal values are summarised in Table 3. The mean concentrations were within 8% below and 20% above nominal values, confirming the accuracy of formulation.

Individual analytical results and associated procedural recovery data are detailed in Table 4. One sample result from the Week 12, Group 3 samples was excluded from the mean as considered an outlier. It was not possible to perform additional confirmatory analysis as microbial growth was observed in the formulation and the result was therefore reported as the result of one analysis. Procedural recovery values were between 84.2% and 100.3% of the fortified concentration, reflecting the complexity of the analytical method.

CONCLUSION

The analytical procedure was validated with respect to accuracy and precision at nominal concentrations in the range 50 mg/mL to 200 mg/mL.

The glucose content of ABM-FD in aqueous formulations was confirmed as consistent at nominal concentrations of 50 mg/mL and 200 mg/mL during storage at ambient temperature for 1 day and refrigerated storage for 8 days. The storage times represented the maximum time from preparation to completion of administration.

The mean concentrations of ABM-FD, analysed with respect to glucose content, in test formulations were within ±20% of nominal concentrations.

TABLES

1. Method validation for ABM-FD in aqueous formulation

Analytical phase	Nominal fortification, mg/mL		
	50	100	200
Day 1 stability	93.9	-	96.5
Week 1	84.2	100.2	94.0
Day 8 stability	106.0	-	92.6
Week 12	93.6	88.1	100.3
Mean (%)	94.9		
CV	6.59		
n	10		

CV Coefficient of variation
n Number of determinations

Results, expressed as percentage recovery, were calculated using the following equation:

$$\%\,\text{Recovery} = \frac{\text{Analysed concentration (mg/mL)}}{\text{Fortified concentration (mg/mL)}} \times 100$$

TABLES
(continued)

2. Glucose content of ABM-FD in aqueous formulations during storage

Nominal inclusion (mg/mL)	Storage conditions			Analysed concentration (mg/mL)			RME (%)	
	Day	Hour	Temp.°C	Analysis 1	Analysis 2	Mean	A	B
50	0	0	+21	59.5	68.3	63.9	+27.8	-
	0	4	+21	67.9	72.6	70.3	+40.6	+10.0
	1	0	+21	65.5	60.1	62.8	+25.6	-1.7
	1	0	+4	61.4	63.2	62.3	+24.6	-2.5
	8	0	+4	54.1	58.9	56.5	+13.0	-11.6
200	0	0	+21	241	276	259	+29.5	-
	0	4	+21	305	306	306	+53.0	+18.1
	1	0	+21	229	243	236	+18.0	-8.9
	1	0	+4	238	229	233	+16.5	-10.0
	8	0	+4	260	265	262	+31.0	+1.2

RME Relative mean error, representing the deviation from A) nominal and B) time zero

Analysed concentrations were calculated using unrounded figures.

TABLES
(continued)

3. Summary: mean concentrations of ABM-FD in test formulations

Week of formulation	Group	Nominal inclusion (mg/mL)	Mean analysed concentration (mg/mL)	RME (%)
1	Control	0	-	-
	2	50	59.9	+19.8
	3	100	101	+1.0
	4	200	194	-3.5
12	Control	0	-	-
	2	50	53.8	+7.6
	3	100	95.0	-5.0
	4	200	209	+4.5

RME Relative mean error, representing the deviation from nominal

TABLES
(continued)

4. Concentrations of ABM-FD in test formulations (individual values)

Week of formulation	Group	Nominal inclusion (mg/mL)	Analysed concentration (mg/mL)			Procedural recoveries (%)
			Analysis 1	Analysis 2	Mean	
1	Control	0	-	-	-	
	2	50	58.6	61.2	59.9	84.2
	3	100	97.8	105	101	100.2
	4	200	202	185	193	94.0
12	Control	0	-	-	-	
	2	50	43.3	64.4	53.8	93.6
	3	100	95.0	189[1]	95.0	88.1
	4	200	210	208	209	100.3

[1] Sample result excluded from the mean as considered an outlier, attributed to analytical error

Analysed concentrations were calculated using unrounded figures and are corrected for the appropriate recovery at analysis given in this Table.

ANNEX 2

Certificate of analysis

分析試験成績書

第104052261-004号
2004年(平成16年)06月08日

依 頼 者 株式会社 岩出菌学研究所

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財団法人
日本食品分析センター
東京本部 〒151-0052 東京都渋谷区元代々木町52番1号
大阪支所 〒564-0051 大阪府吹田市豊津町3番1号
名古屋支所 〒456-0031 名古屋市中区本大須4丁目5番13号
九州支所 〒812-0031 福岡県福岡市博多区下呉服町1番12号
多摩研究所 〒206-0035 東京都多摩市永山6丁目11番10号
千歳研究所 〒066-0052 北海道千歳市文京2丁目3番

2004年(平成16年)05月20日当センターに提出された上記検体について分析試験した結果は次のとおりです。

分析試験結果

分析試験項目	結果	検出限界	注	方法
β-グルカン	2.9g/100g			酵素法

以 上

本成績書を他に掲載するときは当センターの承認を受けて下さい。

財団法人日本食品分析センター

Certificate of Analysis

Document no. 104052261-004
8th June 2004

Client: Iwade Bacteriology Research Co., Lte.
Sample: ABM-FD

Nippon Food Analysis Centre

A result of analysis of the above sample supplied to the centre on 20th May 2004 was as follows.

Parameter	Result	Limit of Detection	Method
β-glucan	2.9g/100g		Enzyme analysis

EYE RESEARCH CENTRE GLP COMPLIANCE STATEMENT YEAR 2003

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY	TEST TYPE
Huntingdon Life Sciences **Eye Research Centre** **Occold** **Eye** **Suffolk** **IP23 7PX**	**Analytical Chemistry** **Ecosystems** **Environmental Fate** **Environmental Toxicity** **Mutagenicity** **Toxicology** **Phys/Chem Tests**

DATE OF INSPECTION
22nd April 2003

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

Roger G. Alexander
25/7/03

Dr. Roger G. Alexander
Head, UK GLP Monitoring Authority

EYE RESEARCH CENTRE GLP COMPLIANCE STATEMENT 2005

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 2004/9/EC

LABORATORY	TEST TYPE
Huntingdon Life Sciences **Eye Research Centre** **Occold** **Eye** **Suffolk** **IP23 7PX**	**Analytical Chemistry** **Clinical Chemistry** **Ecosystems** **Environmental Fate** **Environmental Toxicity** **Mutagenicity** **Toxicology** **Phys/Chem Testing**

DATE OF INSPECTION

12th April 2005

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of the UK GLP Compliance Programme.

At the time of inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

Bryan J. Wright 9/6/05

Mr. Bryan J. Wright
Head, UK GLP Monitoring Authority

HUNTINGDON RESEARCH CENTRE GLP COMPLIANCE STATEMENT YEAR 2003

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY	TEST TYPE
Huntingdon Life Sciences **Huntingdon Research Centre** **Woolley Road** **Alconbury** **Huntingdon** **Cambs.** **PE28 4HS**	**Analytical Chemistry** **Clinical Chemistry** **Ecosystems** **Environmental Fate** **Environmental Toxicity** **Toxicology** **Phys/Chem Tests**

DATE OF INSPECTION

07th April 2003

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

Roger G. Alexander 1/8/03

Dr. Roger G. Alexander
Head, UK GLP Monitoring Authority

HUNTINGDON RESEARCH CENTRE GLP COMPLIANCE STATEMENT 2005

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 2004/9/EC

LABORATORY

Huntingdon Life Sciences
Huntingdon Research Centre
Woolley Road
Alconbury
Cambridgeshire
PE28 4HS

TEST TYPE

Analytical Chemistry
Clinical Chemistry
Ecosystems
Environmental Fate
Environmental Toxicity
Toxicology
Phys/Chem Testing

DATE OF INSPECTION

7th March 2005

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of the UK GLP Compliance Programme.

At the time of inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

Bryan J Wright
9/6/05

Mr. Bryan J. Wright
Head, UK GLP Monitoring Authority