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APPENDICE

APPENDIX A

Calculation of % Net peptide content

$$\% \text{ Peptide content} = \frac{A_{SMP} \times M_R \text{ (mg)} \times V_{SPM} \text{ (ml)} \times 100}{A_R \times M_{SMP} \text{ (mg)} \times V_R \text{ (ml)}} \quad \text{...Eq. I}$$

$$\% \text{ Assay (as is)} = \frac{\% \text{ Net peptide content} \times 100}{(100 - H_2O - \text{AcOH})} \quad \text{...Eq. II}$$

Where:

A_{SMP}	=	peak area of salmon CT in the sample chromatogram		
M_R	=	mass of reference substance used in preparing reference solution; declared content of $C_{145}H_{240}N_{44}O_{48}S_2$ in salmon calcitonin EPCRS vial (1.00 mg no water and acetic acid)	=	1.00 mg
V_{SPM}	=	volume of sample solution (µl)	=	50 µl
100	=	conversion factor to percent	=	100
A_R	=	peak area of reference chromatogram		
M_{SMP}	=	mass of salmon CT used in sample solution (mg)		
V_R	=	volume of reference solution (µl)	=	50 µl
H_2O*	=	water content of the test substance	=	4.2% w/w
AcOH**	=	acetic acid content of the test substance	=	11.0% w/w

*, ** Taken from Bachem's certificate of analysis for salmon CT, Lot No. 0547992 (as see in Appendix B)

Table 50 Individual Peak Area of analysis for salmon CT *EPCRS*

No. of Injection	Peak Area sCT *EPCRS*
1	1817105
2	1811592
3	1831361
4	1886643
5	1839902
Mean	1837320.60
S.D.	29772.10
% C.V.	1.62

Example; when peak area of salmon CT (Bachem®) injection no.1 = 1930306

Mass of salmon calcitonin of sample = 1.232mg

$$\% \text{ Peptide content} = \frac{1930306 \times 1.00\ (\text{mg}) \times 50(\mu\text{l}) \times 100}{1837320.6 \times 1.232\ (\text{mg}) \times 50(\mu\text{l})}$$

$$= 85.28\ \%$$

$$\% \text{ Assay (as is)} = \frac{85.28\ \% \times 100}{(100 - 4.2\% - 11.0\%)}$$

$$= 100.57\ \%$$

Table 51 Peak Area for Five Determination of Salmon CT (Bachem AG, Bubendorf, Switzerland) Lot-No. 0557992.

No. of Injection	Peak Area sCT Bachem®	% Net assay	% Peptide content
1	1930366	85.28	100.57
2	1913894	84.55	99.71
3	1882369	83.16	98.06
4	1895492	83.74	98.75
5	1936055	85.53	100.86
Mean	1911635.20	84.45	99.59
S.D.	22745.25	1.00	1.18
% C.V.	1.19	1.19	1.19

APPENDIX B

Certificate of Analysis of Salmon Calcitonin (Bachem AG, Bubendorf, Switzerland) Lot-No. 0557992. Page 1.

BACHEM

CERTIFICATE OF ANALYSIS *Lot-No.: 0547992*

Product: Calcitonin (salmon I) Ph.Eur. 1997

Formula: $C_{145}H_{240}N_{44}O_{48}S_2$

Molecular weight: 3431.9 g/mol net peptide

Tests	Specifications	Results
Appearance	white to off-white powder, free from visible impurities	complies
Solubility	freely soluble in water	complies
Identification (TLC)	to comply with the approved test	complies
Identification (amino acid analysis)	Arg 0.9 - 1.1 Lys 1.8 - 2.2 Asx 1.8 - 2.2 Pro 1.7 - 2.3 Cys 1.4 - 2.1 Ser 3.2 - 4.2 Glx 2.7 - 3.3 Thr 4.2 - 5.2 Gly 2.7 - 3.3 Tyr 0.7 - 1.1 His 0.9 - 1.1 Val 0.9 - 1.1 Leu 4.5 - 5.3	Arg 1.0 Lys 2.0 Asx 2.0 Pro 2.0 Cys 2.1 Ser 3.5 Glx 3.0 Thr 4.7 Gly 3.0 Tyr 0.9 His 1.0 Val 1.0 Leu 4.9
Identification (ESI-MS)	m = 3432 ± 2 u	m = 3432 u
Absorbance (at 275 nm)	0.40 to 0.55 (corrected for peptide content)	0.44
Absorbance ratio (275 nm/254 nm)	≥ 1.6	2.4
Purity (HPLC)	≥ 99% (TFA-system) ≥ 99% (TEAP-system)	99.6% (TFA-system) 99.6% (TEAP-system)
Water content	≤ 10%	4.2%
Acetic acid content	≤ 15%	11.0%
Total of water and acetic acid	≤ 20%	15.1%
Chloride content	≤ 7%	< 7%
Trifluoroacetic acid content	≤ 0.1%	< 0.04%
Residual organic solvent acetonitrile	≤ 100 ppm	< 100 ppm
Peptide content (CHN)	≥ 82%	84.6%

continued on page 2

Certificate of Analysis of Salmon Calcitonin (Bachem AG, Bubendorf, Switzerland) Lot-No. 0557992. Page 2.

BACHEM

Lot-No.: 0557992
page 2

Tests	Specifications	Results
Bioactivity (as is)	report	6450 I.U./mg
Bioactivity (net peptide)	≥ 5000 I.U. per mg	7622 I.U./mg
Date of Manufacture: Date of Retest:	February 1, 2002 February 2004	
Date: February 13, 2002	Signature: D. Arn D. Arn, Ph.D. Manager Quality Assurance	

APPENDIX C

Table 52 Individual Peak areas of salmon CT standard solutions for construction calibration curve

Standard no.	Known Concentration. (µg /ml)	Estimate Concentration (µg/ml)	Peak Area of salmon CT				S.D.	% C.V.
			Injection no. 1	Injection no. 2	Injection no. 3	Mean		
1	40.0	43.20	1933509	1931872	1932946	1932775.67	831.69	0.04
2	30.0	32.40	1416091	1426668	1460646	1434468.33	23279.19	1.62
3	20.0	21.60	936932	919499	954900	937110.33	17701.17	1.89
4	10.0	10.80	425012	427785	432709	428502.00	3898.27	0.91
5	5.0	5.40	197037	199638	196961	197878.67	1524.10	0.77
6	2.5	2.70	88270	88607	89486	88787.67	627.81	0.71
7	1.0	1.08	26921	26823	27799	27181.00	537.44	1.98

*The linear regression equation for this curve was Y = 45516 X - 40981

Table 53 Individual peak areas of salmon CT standard solutions for accuracy data.

Std no.	Actual Std. Conc. (µg/ml)	Peak area of Salmon CT standard					Mean	S.D.	% C.V.
		Replicate no. 1	Replicate no. 2	Replicate no. 3	Replicate no. 4	Replicate no. 5			
1	40.64	1853082	1801696	1856096	1837539	1862648	1842212.20	24453.71	1.33
2	20.32	946372	919499	930993	954900	936932	937739.20	13673.75	1.46
3	10.16	425012	427785	428502	432813	437709	430364.20	4967.62	1.15
4	5.08	197948	199157	196778	195387	191901	196234.20	2795.98	1.42
5	1.02	32490	32754	33945	33602	32431	33044.40	687.38	2.08
1		40.74	39.62	40.80	40.40	40.95	40.50	0.53	1.31
2	Interpolated	21.03	20.45	20.70	21.22	20.83	20.84	0.30	1.43
3	conc.	9.70	9.76	9.77	9.87	9.97	9.82	0.11	1.10
4	(µg/ml)	4.76	4.79	4.74	4.71	4.63	4.73	0.06	1.29
5		1.17	1.17	1.20	1.19	1.17	1.18	0.01	1.27
1		100.24	97.49	100.40	99.41	100.75	99.66	1.31	1.31
2	%	103.50	100.62	101.85	104.41	102.49	102.57	1.46	1.43
3	Analytical	95.46	96.05	96.21	97.13	98.18	96.61	1.06	1.10
4	Recovery	93.77	94.29	93.27	92.68	91.19	93.04	1.20	1.29
5		114.47	115.04	117.57	116.84	114.35	115.66	1.46	1.27

$$\% \text{ Recovery} = \frac{\text{Interpolated concentration}}{\text{Actual concentration}} \times 100$$

(n = 5 replicate/conc)

Table 54 Individual peak area of salmon CT standard solutions for within-run precission

Standard no.	Known Concentration (mcg/ml)	Peak Area					Mean	S.D.	C.V.
		assay 1	assay 2	assay 3	assay 4	assay 5			
1	40.0	1930366	1913894	1882369	1895492	1936055	***1911635.2***	***22745.2***	***1.19***
2	10.0	370102	368361	369327	362099	376778	***369333.4***	***5227.21***	***1.42***
3	1.0	39437	39263	38625	38772	40133	***39246***	***598.719***	***1.53***

Table 55 Individual peak area of salmon CT standard solutions for between-run precision.

Standard no.	Known Concentration (mcg/ml)	Peak Area					Mean	S.D.	C.V.
		assay 1	assay 2	assay 3	assay 4	assay 5			
1	40.0	1856096	1862648	1815308	1811186	1837539	***1836555.4***	***23230.82***	***1.26***
2	10.0	443325.3	440162	451282	438532	456443	***445948.87***	***7647.71***	***1.71***
3	1.0	40254	40553	38904	39941	40649	***40060.2***	***703.23***	***1.76***

APPENDIX D

Calculation

1. Mean ($\overline{X}$)

$$\overline{X} = \sum X / n$$

2. Standard deviation(S.D.)

$$S.D. = \sqrt{\sum (X - \overline{X})^2 / n-1}$$

3. Coefficient of variation (C.V.)

$$C.V. = (S.D. / \overline{X})$$

4. Area under the plasma drug concentration time curve (AUC_{0-t})

$$AUC_{o-t} = \frac{\sum (C_{n-1} + C_{n-1})(t_n - t_{n-1})}{2}$$

5. Area under the plasma drug concentration time curve ($AUC_{0-\infty}$)

$$AUC_{o-\infty} = \frac{\sum (C_{n-1} + C_{n-1})(t_n - t_{n-1})}{2} + \hat{C}/K_e$$

Where; $\hat{C}$ = The last measurable plasma drug concentration

K_e = Elimination rate constant

6. Elimination rate constant (K_e)

$$K_e = \frac{\ln C_1 - \ln C_2}{t_2 - t_1}$$

7. Elimination half life($t_{1/2}$)

$$t_{1/2} = 0.693 / K_e$$

8. Analysis of variance for two way crossover design

The experimental design is

Sequence	Subject no.	Period	
		I	II
I	1,2,3,4,5,6	A	B
II	7,8,9,10,11,12	B	A

Where; A = Innovator's product

B = Test product

In statistical terms the calculations to set up an analysis of variance table are as follows:

Sorce of variation	df	Sum of square	Mean Square
Total	2n-1	SSTO	
sequences	g-1	SSG	MSG=SSG/DF
Subjects c in sequence	n-2	SSS	MSS=SSS/DF
Periods	p-1	SSP	MSP=SSF/DF
Formulation	f-1	SSF	MSF=SSF/DF
Error	n-2	SSE	MSE=SSE/DF

Where; n = Number of subjects

SSTO = Sum of square total

SSG = Sum of square sequence

SSS = Sum of square subject

SSF = Sum of square formulation

SSP = Sum of square period

SSE = Sum of square error

df = degree of freedom

g = number of groups

n1 = number of subjects in sequence 1

n2 = number of subjects in sequence 2

p = number of periods

f = number of formulations

APPENDIX E

NO. 141/ 2003

Study Protocol Approval

The Ethics Committee of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand has approved the following study to be carried out according to the protocol dated and/ or amended as follows :

Study Title : Formulation, Stability and Bioequivalence of Salmon Calcitonin Nasal Sprays

Study Code : -

Centre : Chulalongkorn University

Principal Investigator : Miss Bordeesuda Suiwongsa

Protocol Date : January 22, 2003

A list of the Ethics Committee members and positions present at the Ethics Committee meeting on the date of approval of this study has been attached.

This Study Protocol Approval Form will be forwarded to the Principal Investigator.

Chairman of Ethics Committee : .....

(Signature)

Boonyong Tantisirs, Ph.D.

Secretary of Ethics Committee : .....

(Signature)

Poj Kulvanich, Ph.D.

Date of Approval : August 19, 2003

หนังสือแสดงความยินยอม

การวิจัยเรื่อง การตั้งตำรับ ความคงตัวและชีวสมมูลของยาพ่นจมูกแซลมอนแคลซิโตนิน

วันให้คำยินยอม วันที่ .....เดือน..... พ.ศ. 2547

ข้าพเจ้า(นาย/นาง/นางสาว).....นามสกุล.....
อยู่บ้านเลขที่.....ซอย.....ถนน.....แขวง/ตำบล.....
เขต/อำเภอ.....จังหวัด.....รหัสไปรษณีย์.....

ก่อนที่จะลงนามในใบยินยอมให้ทำการวิจัยนี้ ข้าพเจ้าได้รับเอกสารและอธิบาย จากผู้วิจัยให้ทราบวัตถุประสงค์ของการวิจัย วิธีวิจัย อันตรายหรืออาการข้างเคียงที่อาจเกิดขึ้นจากการวิจัยหรือจากยาที่ใช้รวมทั้งประโยชน์ที่จะเกิดขึ้นจากการวิจัยอย่างละเอียด และมีความเข้าใจดีแล้ว

ผู้วิจัยได้ตอบคำถามต่างๆที่ข้าพเจ้าสงสัยด้วยความเต็มใจ ไม่ปิดบังซ่อนเร้นจนข้าพเจ้าพอใจ

ข้าพเจ้าเข้าร่วมโครงการนี้โดยสมัครใจ และมีสิทธิ์ที่จะบอกเลิกการเข้าร่วมโครงการวิจัยนี้เมื่อใดก็ได้ โดยการบอกเลิก จะไม่มีผลต่อการรักษาโรคที่ข้าพเจ้าจะได้รับต่อไป

ข้าพเจ้าอนุญาตให้ผู้วิจัยเปิดเผยข้อมูลเกี่ยวกับตัวข้าพเจ้าในหน่วยงานที่เกี่ยวข้องได้ตามที่ผู้วิจัยเห็นสมควร ผู้วิจัยรับรองว่า จะเก็บข้อมูลเฉพาะเกี่ยวกับตัวข้าพเจ้าเป็นความลับ และจะเปิดเผยได้เฉพาะในรูปที่เป็นสรุปผลการวิจัย

ในการวิจัยครั้งนี้จะมีการเจาะเลือดเป็นจำนวน 7 ซีซี ทุกเวลา 0 ,5,10,15,20,30,45,60, 90,120,180 และ240 นาที เป็นจำนวน 12 ครั้ง

ผู้วิจัยได้อธิบายให้ข้าพเจ้าทราบและเข้าใจแล้วว่า การเจาะเลือดเพียงเล็กน้อย โดยทั่วไปจะไม่เกิดอันตรายใดๆแก่ข้าพเจ้าเลย นอกจากอาจมีรอยช้ำบริเวณที่เจาะเล็กน้อย ซึ่งอาจหายได้เองใน 7 วัน

ผู้วิจัยรับรองว่า หากเกิดอันตรายใดๆ จากการวิจัยดังกล่าว ข้าพเจ้าจะได้รับการรักษาพยาบาลโดยไม่คิดมูลค่า และจะได้รับการชดเชยรายได้ที่สูญเสียไประหว่างการรักษาพยาบาลดังกล่าว ตลอดจนเงินทดแทนความพิการที่อาจจะเกิดขึ้น และรายละเอียดเกี่ยวกับการรักษาพยาบาลหรือเงินชดเชยดังกล่าวข้าพเจ้าสามารถติดต่อได้ที่.....
โดยบุคคลที่รับผิดชอบเรื่องนี้คือ ร.ศ. ดร. ภาคภูมิ เต็งอำนวย

ข้าพเจ้าได้อ่านข้อความข้างต้นแล้ว และมีความเข้าใจดีทุกประการ จึงได้ลงนามในใบยินยอมนี้ด้วยความเต็มใจ

ลงนาม.....ผู้ยินยอม

ลงนาม.....ผู้รับผิดชอบการวิจัย

ลงนาม.....พยาน

ลงนาม.....พยาน

แบบบันทึกอาการอันไม่พึงประสงค์จากการใช้ยา Calcitonin Nasal Spray

(Case Record Form)

ชื่อโครงการวิจัย: ชีวสมมูลของยาพ่นจมูกแคลซิโตนิน

ชื่อ/นามสกุลอาสาสมัคร : .....อายุ.....ปี เพศ.....

น้ำหนัก.....กก.

ประวัติการแพ้ยา: ☐ ไม่มี ☐ มี (ระบุ).....

การศึกษาครั้งที่ .....วัน/เดือน/ปี 31 ม.ค. 2547...ได้รับยารหัส.....ขนาด .....200 IU

ชื่อ/นามสกุลแพทย์ผู้ดูแล: นพ. จตุพร โชติกวณิชย์

อาการไม่พึงประสงค์: ☐ พบ ☐ ไม่พบ

อาการที่พบหรือปรากฏ	เวลาหลังจากได้รับยา	วัน/เดือน/ปี

ความรุนแรง : ☐ น้อย ☐ ปานกลาง ☐ มาก

ภายหลังเกิดอาการ: ☐ ให้รักษาทันที ☐ เฝ้าระวังอาการ ☐ ให้ถอนตัว

☐ ให้ทดลองต่อ ☐ อื่นๆ (ระบุ).....

ผลลัพธ์ที่เกิดขึ้น: ☐ หายเป็นปกติ ☐ ยังมีอาการอยู่ ☐ ไม่สามารถติดตามผล

ผลการประเมินความสัมพันธ์ของยา Calcitonin Nasal Spray กับอาการไม่พึงประสงค์

☐ ใช่แน่นอน ☐ น่าจะใช่ ☐ อาจจะใช่ ☐ สงสัย

หมายเหตุ: .....

ลงชื่อ.....

(นพ. จตุพร โชติกวณิชย์)

ผู้วินิจฉัยอาการ/ผู้ประเมินและบันทึก

ลงชื่อ.....

(รศ.ดร. ภาคภูมิ เต็งอำนวย)

ผู้วิจัยหลัก

Table 56 Demographic Data of Subjects Participated in This Study

Subject no.	Age (yr)	Height (m)	Weight (kg)	BMI (kg/m^2)
1	39	1.67	55	19.72
2	25	1.66	57	20.69
3	23	1.75	73	23.84
4	28	1.65	74	27.18
5	30	1.67	60	21.51
6	48	1.66	65	23.59
7	23	1.73	68	22.72
8	26	1.65	59	21.67
9	25	1.68	64	22.68
10	35	1.71	57	19.49
11	36	1.65	70	25.71
12	25	1.69	55	19.26
Mean	30.25	1.68	63.08	22.34
S.D.	7.74	0.03	6.91	2.47
%C.V.	25.57	1.97	10.95	11.06

$$\text{Body mass index (BMI)} = \frac{\text{Weight (kg)}}{\text{Height (m}^2\text{)}}$$

Table 57 Blood Chemical tests and of subjects Participated in This Study

Blood chemical test	Normal range	Subject no. 1	2	3	4	5	6	7	8	9	10	11	12
CBC	Normal	N	N	N	N	N	N	N	N	N	N	N	N
FBS	70 -110mg/dL	N	N	N	N	N	N	N	N	N	N	N	N
		(101)	(88)	(93)	(92)	(110)	(96)	(102)	(*)	(*)	(91)	(101)	(90)
BUN	8-20 mg/dL	N	N	N	N	N	N	N	N	N	N	N	N
		(*)	(13)	(11)	(*)	(4)	(*)	(8)	(*)	(*)	(*)	(19)	(*)
Serum creatinine	0.7-1.5mg/dL	N	N	N	N	N	N	N	N	N	N	N	N
		(*)	(*)	(*)	(*)	(*)	(*)	(*)	(*)	(*)	(*)	(*)	(*)
AST(SGOT)	5.0-50 U/L	N	N	N	N	N	N	N	N	N	N	N	N
		(*)	(40)	(24)	(*)	(48)	(*)	(37)	(*)	(*)	(*)	(20)	(*)
ALT(SGPT)	5.0-45 U/L	N	N	N	N	N	N	N	N	N	N	N	N
		(*)	(24)	(14)	(*)	(31)	(*)	(26)	(*)	(*)	(*)	(19)	(*)
Total bilirubin	0.3-1.0mg/dL	N	N	N	N	N	N	N	N	N	N	N	N
		(*)	(*)	(*)	(*)	(*)	(*)	(*)	(*)	(*)	(*)	(*)	(*)
Alkaline phosphatase	25 - 90 U/L	N	N	N	N	N	N	N	N	N	N	N	N
HBS Ag	Negative	-	-	-	-	-	-	-	-	-	-	-	-

N = Normal

HBSAg = Antibody Hepatitis B

BUN = Blood Urea Nitrogen

AST = Aspartate Aminotransferase

ALT = Alanine Aminotranferase

\- = Negative

* = Report as normal without provision of numerical value

Table 58 Typical RIA Standard Curve for Determination of Plasma Salmon Calcitonin.

Tube No.	Tube label	Concentration (pg/ml)	Mean* (cpm)	subtract NSB	B/Bo (%)
1,2	TC		25075.60	24617.90	
3,4	NSB		457.70	0.00	
5,6	Standard A	0.0	6337.30	5879.60	100
7,8	Standard B	7.5	5781.25	5323.55	90.54
9,10	Standard C	30.0	5413.80	4956.10	84.29
11,12	Standard D	60.0	4114.75	3657.05	62.2
13,14	Standard E	125.0	2819.15	2361.45	40.16
15,16	Standard F	250.0	1966.55	1508.85	25.66
17,18	Standard G	500.0	867.90	410.20	6.98

* Each value is mean of two determinations.

Table 59 Determination of reference standard salmon CT Level I,II and III

Tube label	Concentration (pg/ml)	cpm	Extrapolated concentration (pg/ml)	Average	S.D.	% C.V.
Level I	35±10	4962.30	34.53			
Level I	35±10	5006.00	32.68	*33.61*	*1.31*	*3.91*
Level II	80±25	3670.50	99.53			
Level II	80±25	3868.90	88.00	*93.76*	*8.15*	*8.69*
Level III	180 ± 55	2153.80	222.37			
Level III	180 ± 55	2360.00	200.31	*211.34*	*15.60*	*7.38*

Table 60 Logarithmically transformed of pharmacokinetic parameters (AUC_{0-t}, $AUC_{0-\infty}$ and C_{max}) of 12 subjects following intranasal administration of the Test's product.

Subject	Ln AUC_{0-t}	Ln $AUC_{0-\infty}$	Ln C_{max}
1	7.96	8.15	4.71
2	7.79	7.86	4.89
3	8.18	8.21	4.63
4	8.29	8.30	4.85
5	8.25	8.26	4.86
6	7.83	7.84	4.73
7	8.25	8.29	4.90
8	7.99	8.08	4.88
9	8.09	8.26	4.78
10	7.90	7.95	4.70
11	7.92	8.00	4.88
12	7.81	7.84	4.77
Mean	8.02	8.09	4.80
S.D.	0.18	0.18	0.09
%C.V.	2.30	2.25	1.85

Table 61 Logarithmically transformed of pharmacokinetic parameters (AUC_{0-t}, $AUC_{0-\infty}$ and C_{max}) of 12 subjects following intranasal administration of the Innovator's product

Subject	Ln AUC_{0-t}	Ln $AUC_{0-\infty}$	Ln C_{max}
1	7.80	8.15	4.87
2	7.86	7.86	4.90
3	7.99	8.21	4.78
4	8.40	8.30	4.83
5	8.22	8.26	4.92
6	7.81	7.84	4.72
7	8.15	8.29	4.79
8	7.92	8.08	4.69
9	7.89	8.26	4.84
10	8.00	7.96	4.88
11	7.99	8.00	4.89
12	8.00	7.85	4.86
Mean	8.00	8.09	4.83
S.D.	0.18	0.18	0.07
%C.V.	2.23	2.25	1.48

Data presented are individual subject of the ln AUC $_{o-t}$ of salmon calcitonin following nasal administration of 400 IU nasal spray. (Innovator's and Test's product)

Sequence	Subject	Innovator's Product		Test' Product		Subject Total
I	1		7.80		7.96	15.76
	2		7.86		7.79	15.65
	3		7.99		8.18	16.17
	4		8.40		8.29	16.69
	5	48.08	8.22	48.30	8.25	16.47
	6		7.81		7.83	15.64
II	7		8.15		8.25	16.40
	8		7.92		7.99	15.91
	9		7.89		8.09	15.98
	10		8.00		7.90	15.90
	11	47.95	7.99	47.96	7.92	15.91
	12		8.00		7.81	15.81
Formulation Total			96.03		96.26	192.29

Period I = 48.08 + 48.30 = 96.38

Period II = 47.95 + 47.96 = 95.91

Correction Term = $(192.29)^2/24$ = 1540.64

SStotal = $[(7.80)^2 + (7.86)^2 + \ldots + (7.81)^2] - CT$ = 0.7186

SSsequence = $[(15.76 + 15.65 + \ldots + 15.64)^2 + (16.40 + 15.91 + \ldots + 15.81)^2]/12 - CT$ = 0.0092

SSsubject = $(15.76)^2 + (15.65)^2 + \ldots + (15.81)^2]/ 2 - 0.0092 - CT$ = 0.6164

SSperiod = $[(96.03)^2 + (96.26)^2] /12 - CT$ = 0.0018

SSformulation = $[(96.02)^2 + (96.27)^2] /12 - CT$ = 0.0022

SSerror = 0.7186-0.0092-0.6164 -0.0018-0.0022 = 0.0889

Analysis of Two Way cross-over design

Source of variation	d.f.	Sum of square	Mean Square	F_{ratio}	F_{tab}	Sig Level
Total	23	0.7186	--	--	--	--
sequences	1	0.0092	0.0092	0.15	4.96	NS
Subjects (sequence)	10	0.6164	0.0616	6.93	2.98	S
Period	1	0.0018	0.0018	0.21	4.96	NS
Formulation	1	0.0022	0.0022	0.25	4.96	NS
Error	10	0.0889	0.00889	--	--	--

Data presented are individual subject of the ln AUC $_{0-\infty}$ of salmon calcitonin following nasal administration of 400 IU nasal spray. (Innovator's and Test's product)

Sequence	Subject	Innovator's Product		Test' Product		Subject Total
I	1		7.88		8.15	16.03
	2		7.97		7.86	15.83
	3		8.08		8.21	16.29
	4		8.48		8.30	16.78
	5	48.51	8.28	48.62	8.26	16.54
	6		7.82		7.84	15.66
II	7		8.22		8.29	16.51
	8		7.93		8.08	16.01
	9		7.92		8.26	16.18
	10		8.07		7.95	16.02
	11	48.18	8.01	48.42	8.00	16.01
	12		8.03		7.84	15.87
Formulation Total			96.69		97.04	193.73

Period I = 48.51 + 48.62 = 96.38

Period II = 48.18 + 48.42 = 95.91

Correction Term = $(193.73)^2/24$ = 1563.80

SStotal = $[(7.88)^2 + (7.97)^2 + \ldots + (7.84)^2] - CT$ = 0.7594

SSsequence = $[(16.03 + 15.83 + \ldots + 15.66)^2 + (16.51 + 16.01 + \ldots + 15.87)^2]/12 - CT$ = 0.0117

SSsubject = $(16.03)^2 + (15.83)^2 + \ldots + (15.87)^2]/2 - 0.0117 - CT$ = 0.5833

SSperiod = $[(96.93)^2 + (96.80)^2]/12 - CT$ = 0.0007

SSformulation = $[(96.69)^2 + (97.04)^2]/12 - CT$ = 0.0051

SSerror = 0.7594 - 0.0117 - 0.5833 - 0.0007 - 0.0051 = 0.1585

Analysis of Two Way cross-over design

Source of variation	d.f.	Sum of square	Mean Square	F_{ratio}	F_{tab}	Sig Level
Total	23	0.7594	--	--	--	--
sequences	1	0.0117	0.0117	0.20	4.96	NS
Subjects (sequence)	10	0.5833	0.0583	3.68	2.98	S
Periods	1	0.0007	0.0007	0.04	4.96	NS
Formulation	1	0.0051	0.0051	0.32	4.96	NS
Error	10	0.1585	0.0159	--	--	--

Data presented are individual subject of the ln C_{max} of salmon calcitonin following nasal administration of 400 IU nasal spray. (Innovator's and Test's product)

Sequence	Subject	Innovator's Product		Test' Product		Subject Total
I	1		4.87		4.71	9.58
	2		4.90		4.89	9.79
	3		4.78		4.63	9.41
	4		4.83		4.85	9.68
	5	29.02	4.92	28.67	4.86	9.78
	6		4.72		4.73	9.45
II	7		4.79		4.90	9.69
	8		4.69		4.88	9.57
	9		4.84		4.78	9.62
	10		4.88		4.70	9.58
	11	28.95	4.89	28.91	4.88	9.77
	12		4.86		4.77	9.63
Formulation Total			57.97		57.58	115.55

Period I = 29.02 + 28.67 = 57.69

Period II = 28.95 + 28.91 = 57.86

Correction Term = $(115.55)^2/24$ = 556.33

SStotal = $[(4.87)^2+ (4.90)^2 +...+(4.77)^2] - CT$ = 0.1540

SSsequence = $[(9.58 + 9.79 +...+9.45)^2 + (9.69 +9.57+...$
$+9.63)^2]/12 -CT$ = 0.0012

SSsubject = $(9.58)^2 + (9.79)^2 +...+(9.63)^2]/ 2- 0.0012-CT$ = 0.0804

SSperiod = $[(57.93)^2 +(57.62)^2] /12 - CT$ = 0.0040

SSformulation = $[(57.97)^2 +(57.58)^2] /12 - CT$ = 0.0063

SSerror = 0.1540 -0.0012 – 0.0804 – 0.0040 – 0.0063 = 0.0620

Analysis of Two Way cross-over design

Source of variation	d.f.	Sum of square	Mean Square	F_{ratio}	F_{tab}	Sig Level
Total	23	0.1540	--	--	--	--
sequences	1	0.0012	0.0012	0.15	4.96	NS
Subjects (sequence)	10	0.0804	0.0080	1.30	2.98	S
Periods	1	0.0040	0.0040	0.65	4.96	NS
Formulation	1	0.0063	0.0063	1.02	4.96	NS
Error	10	0.0620	0.00620	--	--	--

Data presented are individual subject of the ln Ke of salmon calcitonin following nasal administration of 400 IU nasal spray. (Innovator's and Test's product)

Sequence	Subject	Innovator's Product		Test' Product		Subject Total
I	1		-3.85		-3.85	-7.58
	2		-4.04		-4.00	-8.04
	3		-3.73		-3.51	-7.24
	4		-4.16		-3.92	-8.08
	5	-22.68	-3.66	-22.76	-3.61	-7.27
	6		-3.24		-3.87	-7.11
II	7		-4.03		-3.66	-7.69
	8		-3.47		-4.01	-7.48
	9		-3.51		-4.22	-7.74
	10		-4.09		-3.83	-7.92
	11	-22.97	-3.83	-22.72	-3.46	-7.30
	12		-4.03		-3.54	-7.57
Formulation Total			-45.65		-45.49	-91.13

Period I = (-22.68) + (-22.76) = -45.44

Period II = (-22.97) + (-22.72) = -45.69

Correction Term = $(-91.13)^2/24$ = 346.06

SStotal = $[(-3.85)^2 + (-4.04)^2 + \ldots + (-3.54)^2] - CT$ = 1.517

SSsequence = $[(-7.70 + -8.04 \ldots + -7.11)^2 + (-7.69 + -7.48 + \ldots + -7.58)^2]/12 - CT$ = 0.0026

SSsubject = $(-7.70)^2 + (-8.04)^2 + \ldots + (-7.57)^2]/2 - 0.0026 - CT$ = 0.5734

SSperiod = $[(-45.40)^2 + (45.73)^2]/12 - CT$ = 0.0046

SSformulation = $[(-45.65)^2 + (-45.48)^2]/12 - CT$ = 0.0011

SSerror = 1.517-0.0026-0.5760-0.0046-0.0011 = 0.9354

Analysis of Two Way cross-over design

Source of variation	d.f.	Sum of square	Mean Square	F_{ratio}	F_{tab}	Sig Level
Total	23	1.5170		--	--	--
sequences	1	0.0026	0.0026	0.04	4.96	NS
Subjects (sequence)	10	0.5760	0.0576	0.62	2.98	NS
Periods	1	0.0046	0.0046	0.05	4.96	NS
Formulation	1	0.0011	0.0011	0.01	4.96	NS
Error	10	0.9354	0.0935	--	--	--

Data presented are individual subject of the ln t 1/2 of salmon calcitonin following nasal administration of 400 IU nasal spray. (Innovator's and Test's product)

Sequence	Subject	Innovator's Product		Test' Product		Subject Total
I	1		3.49		3.48	6.97
	2		3.68		3.63	7.31
	3		3.36		3.15	6.51
	4		3.79		3.55	7.34
	5	20.48	3.29	20.55	3.24	6.53
	6		2.87		3.50	6.37
II	7		3.67		3.29	6.96
	8		3.10		3.64	6.74
	9		3.15		3.86	7.01
	10		3.73		3.46	7.19
	11	20.78	3.47	20.53	3.10	6.57
	12		3.66		3.18	6.84
Formulation Total			41.26		41.08	82.34

Period I = $20.48 + 20.55 = 41.03$

Period II = $20.78 + 20.53 = 41.31$

Correction Term = $(82.34)^2/24$ = 282.49

SStotal = $[(3.49)^2 + (3.68)^2 + \ldots + (3.18)^2] - CT$ = 1.5204

SSsequence = $[(6.97 + 7.31 + \ldots + 6.37)^2 + (6.96 + 6.74 + \ldots + 6.84)^2]/12 - CT$ = 0.0033

SSsubject = $(6.97)^2 + (7.31)^2 + \ldots + (6.84)^2]/2 - 0.0033 - CT$ = 0.5751

SSperiod = $[(41.01)^2 + (41.33)^2]/12 - CT$ = 0.0043

SSformulation = $[(41.26)^2 + (41.08)^2]/12 - CT$ = 0.0014

SSerror = $1.5204 - 0.0033 - 0.5784 - 0.0043 - 0.0014$ = 0.9364

Analysis of Two Way cross-over design

Sorce of variation	df	Sum of square	Mean Square	F cal	Ftab	Sig Level
Total	23	1.5204	--	--	--	--
sequences	1	0.0033	0.0033	0.06	4.96	NS
Subjects (sequence)	10	0.5784	0.0578	0.62	2.98	NS
Periods	1	0.0043	0.0043	0.05	4.96	NS
Formulation	1	0.0014	0.0014	0.01	4.96	NS
Error	10	0.9364	0.0936	--	--	--

APPENDIX F

Table 62 Peak area of CT and N-acetyl-cys[1]-calcitonin for assay percent of related peptide

A. Day 0 (Test and Innovator's Product)

	Retention time (min)		Relative retention time	Peak area		Peak Area Total	% Related Peptide by total area
	Calcitonin	N-acetyl-cys[1]-calcitonin		Calcitonin	N-acetyl-cys[1]-calcitonin		
Batch I	23.313	25.915	1.112	990277	8661	998938	0.87
Batch II	23.243	25.820	1.111	1022410	8576	1030986	0.83
Batch III	22.442	25.123	1.119	2021458	22276	2043735	1.09
Batch IV	22.634	25.353	1.120	1960640	22030	1982670	1.11
Miacalcic®	22.276	25.218	1.132	2025242	19793	2045035	0.97

B. 30°C 4 months

	Retention time (min)		Relative retention time	Peak area		Peak Area Total	% Related Peptide by total area
	Calcitonin	N-acetyl-cys[1]-calcitonin		Calcitonin	N-acetyl-cys[1]-calcitonin		
Batch I	22.739	25.212	1.109	980887	11923	992810	1.20
Batch II	22.371	24.920	1.114	940923	14923	955846	1.56
Batch III	22.324	24.937	1.117	1973486	29901	2003387	1.49
Batch IV	22.612	25.163	1.113	1905125	27460	1932585	1.42

C.4°C 6months

	Retention time (min)		Relative retention time	Peak area		Peak Area Total	% Related Peptide by total area
	Calcitonin	N-acetyl-cys[1]-calcitonin		Calcitonin	N-acetyl-cys[1]-calcitonin		
Batch I	22.894	25.771	1.126	696686	13132	709818	1.85
Batch II	22.857	25.762	1.127	698652	13226	711878	1.86
Batch III	23.072	25.217	1.093	1396500	37220	1433720	2.60
Batch IV	22.634	25.353	1.120	1409368	37293	1446661	2.58

$$\text{Relative retention} = \frac{\text{retention time of N-acetyl-cys[1]-calcitonin}}{\text{retention time of CT}}$$

$$\text{Peak area total} = \text{Peak area of CT} + \text{Peak area of N-acetyl-cys[1]-calcitonin}$$

$$\text{\% Related Peptide} = \frac{\text{Peak area of N-acetyl-cys[1]-calcitonin} \times 100}{\text{Peak area total}}$$

Table 63 Peak area of CT and Calcitonin C for determination percent of Calcitonin C.

A. 30 °C 1 mo.

	Retention time (min)		Relative retention time	Peak area		Peak Area Total	% Calcitonin C
	CT	Calcitonin C		CT	Calcitonin C		
Batch I	9.181	16.465	1.79	768663	7386	776049	0.95
Batch II	9.200	16.485	1.79	788696	8135	796831	1.02
Batch III	9.161	16.438	1.79	1546828	15482	1562310	0.99
Batch IV	9.159	16.447	1.80	1549960	14324	1564284	0.92

B. 30 °C 2 mo.

	Retention time (min)		Relative retention time	Peak area		Peak Area Total	% Calcitonin C
	CT	Calcitonin C		CT	Calcitonin C		
Batch I	7.900	15.075	1.91	701575	14704	716279	2.05
Batch II	7.911	15.084	1.91	699915	12498	712413	1.75
Batch III	7.917	15.059	1.90	1441864	29653	1471517	2.02
Batch IV	7.883	15.030	1.91	1425191	27536	1452727	1.90

C. 30 °C 3 mo.

	Retention time (min)		Relative retention time	Peak area		Peak Area Total	% Calcitonin C
	CT	Calcitonin C		CT	Calcitonin C		
Batch I	6.914	14.301	2.07	666429	26232	692661	3.79
Batch II	6.919	14.308	2.07	662906	25539	688445	3.71
Batch III	6.879	14.235	2.07	1391859	51020	1442879	3.54
Batch IV	6.862	14.243	2.08	1370705	48561	1419266	3.42

D. 30 °C 4 mo.

	Retention time (min)		Relative retention time	Peak area		Peak Area Total	% Calcitonin C
	CT	Calcitonin C		CT	Calcitonin C		
Batch I	8.693	15.903	1.83	647910	29234	677144	4.32
Batch II	8.545	15.782	1.85	648566	29473	678039	4.35
Batch III	8.483	14.958	1.76	1350148	61020	1411168	4.32
Batch IV	8.055	15.340	1.90	1340026	60994	1401020	4.35

$$\text{Relative retention} = \frac{\text{retention time of Calcitonin C}}{\text{retention time of CT}}$$

$$\text{Peak area total} = \text{Peak area of CT} + \text{Peak area of calcitonin C}$$

$$\%\ \text{Related Peptide} = \frac{\text{Peak area of calcitonin C} \times 100}{\text{Peak area total}}$$

APPENDIX G

STERILITY TESTS

Procedure - Method II is used for the validation of bacteriostasis and fungistais by the direct transfer method. Inoculate two containers of each sterility test medium with less than 100 colonies forming units, using the volume of medium for each appropriate microorganism specified in Table 64. Add the specified portion of the article under test to

one of the inoculated containers of each medium. The other inoculated container is the positive control. Repeat the procedure for each appropriate microorganism, and incubate the containers at the appropriate temperature for not more than 7 days.

Table 64 Test Microorganisms suitable for use in growth promotion test and the validationtests for the Bateriostatic and fungistasis

Medium	Microorganism(strain)	Incubation(7 days)	
		Temparature	Conditions
Fluid thioglycollate	*Staphyllococcus aureus* ATCC 6538	32.5 ± 2.5°	aerobic
	Pseudomonas aeruginosa ATCC 9027	32.5 ± 2.5°	aerobic
	clostridium sporogenes ATCC11437	32.5 ± 2.5°	aerobic
Alternative thioglycolate	*clostridium sporogenes* ATCC 11437	32.5 ± 2.5°	anaerobic
Soy bean casein digest	*Bacillus subtilis* ATCC 6633	22.5 ± 2.5°	aerobic
	Candida albicans ATCC 10231	22.5 ± 2.5°	aerobic
	*Aspergillus niger*ATCC16404	22.5 ± 2.5°	aerobic

Interpretation- If the growth of test organisms in test container is not visually comparable to that of inoculated control container, the article is bacteriostatic or fungistatic. Use the smallest volume of medium in which the growth of test microorganisms in the presence of the article in the presence of the article or not adversely affected.

Number of article to be tested - If the contents of each article are of sufficient quantity, they may be divided so those equal appropriate portions are added to each of

the specimen media (two or more). If each article dose not contain sufficient quantiies for each medium, use twice the number of article in Table 65.

Table 65 Minimum number of articles to be tested in relation to the number of article in the batch.

Number of articles in the batch	Number of article to be tested
Product no intended for injection	
Not more than 200 articles	5% or 2 articles, whichever is greater
More than 200 article	10 articles
Injections	
Not more than 100 articles	10 % or 4 articles whichever is greater
More than 100 but not more than 500 article	10 articles
More than 500 articles	2% or 20 articles

Incubation conditions – Incubate for not less than 14 days at 32.5+ 2.5 C for fluid Thioglycolate Medium or at 22.5 +2.5 C for the Soybean- casein Digest Medium Regardless of the method of sterility testing. Observed the tubes of media on periodic basis over the 14 days of incubation. If the test specimen is positive before 14 days of incubation, further incubation is not necessary (USP24)

Table 66 Quantities of articles for liquid products

Container content (mL)	Minimum volume taken from each product container for each product container (mL)	Minimum volume of each Medium used for direct transfer of volume taken from each container (mL)
Less than 10	1mL, or entire contents if less than 1 mL	15
10 to less than 50	5mL	40
50 to less than 100	10 mL	80

APPENDIX H

Particle Size and Spray Pattern Analysis

Table 67 Individual data of droplet size distribution (placebo A)

NO.	Percentage share at 10.00 μm (%)	D10 (μm)	D50 (μm)	D90 (μm)	Span
1	3.45	14.24	32.57	79.67	2.01
2	2.84	14.70	34.36	301.13	8.34
3	5.24	12.30	26.75	58.52	1.73
4	1.42	16.76	38.90	188.88	4.42
5	4.91	12.93	32.20	194.18	5.63
6	1.35	15.91	29.91	76.74	2.03
7	5.65	12.07	26.54	60.32	1.82
8	2.29	16.10	39.84	399.27	9.62
9	3.80	14.15	34.80	113.36	2.85
10	3.25	14.35	32.81	99.79	2.60
11	3.10	14.18	32.00	226.83	6.65
12	4.11	13.34	29.84	121.21	3.61
13	3.10	14.90	37.78	124.11	2.89
14	1.13	17.40	34.21	78.16	1.78
15	2.30	15.06	32.09	84.74	2.17
16	2.75	14.87	33.39	91.55	2.30
17	4.55	13.01	29.95	114.48	3.39
18	1.86	17.05	44.98	131.21	2.54
19	1.61	17.62	45.24	130.53	2.50
20	2.65	14.70	32.30	87.42	2.25
21	3.04	14.38	32.07	94.13	2.49
22	2.09	16.59	45.44	331.25	6.92
23	3.90	13.62	31.98	344.08	10.33
24	2.88	14.97	35.05	130.99	3.31
25	3.07	14.61	33.97	116.89	3.01
Min	1.13	12.07	26.54	58.52	1.73
Mean	3.05	14.79	34.36	151.18	3.89
Max	5.65	17.62	45.44	399.27	10.33
SD.	1.207	1.52	5.14	95.79	2.53

D10 = 10% of the droplet diameters are smaller than the indicated value

D50 = 50% of the droplet diameters are smaller than the indicated value

D90 = 90% of the droplet diameters are smaller than the indicated value

Table 68 Individual data of droplet size distribution (placebo B)

NO.	Percentage share at 10.00 μm (%)	D10 (μm)	D50 (μm)	D90 (μm)	Span
1	1.86	15.78	33.29	75.02	1.78
2	3.56	14.33	37.47	452.32	11.69
3	4.47	13.26	34.92	222.12	5.98
4	3.34	14.57	38.96	166.37	3.90
5	2.57	14.90	32.14	68.76	1.68
6	2.37	15.35	34.65	87.81	2.09
7	3.65	13.95	36.13	110.29	2.67
8	1.89	15.53	34.01	135.62	3.53
9	2.81	15.43	39.86	111.68	2.41
10	2.53	14.60	31.62	116.62	3.23
11	4.05	13.34	30.36	186.20	5.69
12	2.50	14.96	34.45	127.06	3.25
13	3.29	14.12	32.16	76.50	1.94
14	4.19	13.18	30.40	380.20	12.07
15	2.90	14.90	37.47	506.71	13.13
16	3.18	13.56	27.63	154.11	5.09
17	2.71	14.83	34.22	93.73	2.31
18	2.69	14.62	31.92	83.14	2.15
19	2.36	15.56	37.19	115.93	2.70
20	3.46	14.13	34.46	193.94	5.22
21	4.24	13.60	34.93	290.20	7.92
22	1.99	15.85	39.73	451.14	10.96
23	3.54	14.18	36.13	111.90	2.70
24	2.98	14.20	31.71	316.36	9.53
25	3.68	13.80	32.81	91.99	2.38
Min	1.86	13.18	27.63	68.76	1.68
Mean	3.07	14.50	34.34	189.03	5.04
Max	4.47	15.85	39.86	506.71	13.13
SD.	0.743	0.80	3.07	132.33	3.65

D10 = 10% of the droplet diameters are smaller than the indicated value

D50 = 50% of the droplet diameters are smaller than the indicated value

D90 = 90% of the droplet diameters are smaller than the indicated value

Table 70 Individual data for determination spray pattern analysis (Placebo B)

NO.	Mean Diameter (mm)	Smallest Diameter (mm)	Largest Diameter (mm)	Angle (°)	Ratio (Largest/Smallest)
1	33	37.0	35.0	61.0	1.12
2	34	45.0	39.5	67.0	1.32
3	33	39.0	36.0	62.0	1.18
4	33	41.0	37.0	63.0	1.24
5	36	40.0	38.0	65.0	1.11
6	27	32.0	29.5	52.0	1.19
7	30	42.0	36.0	62.0	1.40
8	26	32.0	29.0	52.0	1.23
9	30	37.0	33.5	58.0	1.23
10	28	40.0	34.0	59.0	1.43
11	33	38.0	35.5	61.0	1.15
12	33	44.0	38.5	65.0	1.33
13	34	37.0	35.5	61.0	1.09
14	30	37.0	33.5	58.0	1.23
15	42	47.0	44.5	73.0	1.12
16	34	48.0	41.0	69.0	1.41
17	30	43.0	36.5	63.0	1.43
18	29	43.0	36.0	62.0	1.48
19	30	37.0	33.5	58.0	1.23
20	30	34.0	32.0	56.0	1.13
21	42	50.0	46.0	75.0	1.19
22	28	35.0	31.5	55.0	1.25
23	38	48.0	43.0	71.0	1.26
24	31	40.0	35.5	61.0	1.29
25	34	40.0	37.0	63.0	1.18
Min	26	32.0	29.0	52.0	1.09
Mean	32.08	39.9	36.0	61.7	1.24
Max	40	49.0	44.0	73.0	1.48
SD.	3.59	3.9	3.3	4.5	0.12

* Dmin = Smallest Diameter
* Dmax = Largest Diameter
* Mean Diameter = [(Dmin + Dmax)/2]
* Spray angle = $180 - 2\theta$,where $\tan\theta = 2h/Dmax$, and

  h=Distance between plate and spray nozzle(h= 30mm)
* Placebo A; representative salmon CT nasal sprays 100 IU per actuation
* Placebo B; representative salmon CT nasal sprays 200 IU per actuation

VITA

Miss Bordeesuda Suiwongsa was born on February 10, 1976 in Bangkok. She received a Bachelor of Pharmacy degree in 1996 from Faculty of Pharmacy, Mahidol University. She is a pharmacist in Pharmacy Department, Siriraj Hospital, Thailand.