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จุฬาลงกรณ์มหาวิทยาลัย

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FORMULATION, STABILITY AND BIOEQUIVALENCE OF SALMON CALCITONIN NASAL SPRAYS

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แคลซิโทนิน(แซลมอน) เป็นเป็ปไทด์ที่ใช้ในการรักษาภาวะกระดูกพรุน และโรคความผิดปกติอื่นของกระดูก ในปัจจุบันยาที่มีใช้ในประเทศไทยอยู่ในรูปแบบยาฉีดและยาพ่นจมูก ซึ่งทั้งสองรูปแบบยังต้องนำเข้าจากต่างประเทศส่งผลให้ยามีราคาแพงและมีการใช้ในวงจำกัด วัตถุประสงค์หลักของการศึกษาครั้งนี้คือต้องการที่จะพัฒนาตำรับยาแซลมอนแคลซิโทนินในรูปแบบยาพ่นจมูกที่มีความคงตัวดี มีชีวประสิทธิผลและมีราคาถูกลงกว่าเดิม ยาพ่นจมูกแซลมอนแคลซิโทนินถูกเตรียมขึ้นเป็นสองความแรงได้แก่ 100 และ 200 ยูนิต ตำรับละสองรุ่นการผลิต ยาพ่นจมูกที่เตรียมได้อยู่ในรูปสารละลายใส มีความเป็นกรดด่างและโทนิซิตี้ที่เหมาะสม บรรจุใส่ขวดพ่นเฉพาะสามารถให้ละอองแต่ละครั้งพ่นเท่ากับ 0.09 มล. ทำการศึกษาความคงตัวในสภาวะเก็บปกติ (4 องศาเซลเซียส) เป็นเวลา 12 เดือนและสภาวะเร่งอุณหภูมิ (30 องศาเซลเซียส) เป็นเวลา 4 เดือน โดยเก็บตัวอย่างมาตรวจตามช่วงเวลาที่กำหนดไว้ เพื่อหาปริมาณตัวยาสำคัญ และนอกเหนือไปจากนั้นได้ทำการหาปริมาณสารสลายตัว(แคลซิโทนิน ซี) พร้อมกับปริมาณของเป็ปไทด์ที่มีโครงสร้างใกล้กัน (เอน-อะเซติล- ซิส-แคลซิโทนิน) โดยใช้เอชพีแอลซี พบว่าปริมาณตัวยาสำคัญของยาพ่นจมูกทุกรุ่นที่ผลิตยังคงอยู่ในช่วงร้อยละ 90 -115 ตลอดช่วงการเก็บตัวอย่าง การทดสอบอื่นได้แก่ ความใส ออสโมลาลิตี้ ความสม่ำเสมอของการให้สเปรย์ และความเป็นกรดด่างของยาพ่นจมูก ตลอดระยะเวลาที่เก็บตัวอย่างพบว่าไม่มีการเปลี่ยนแปลงไปจากค่าเริ่มต้น เพื่อยืนยันในประสิทธิภาพของเครื่องพ่นได้ทำการทดสอบเพิ่มเรื่องการรั่วไหล การกระจายของขนาดละอองสเปรย์และรูปแบบของละอองสเปรย์ การศึกษาชีวประสิทธิผลของยาพ่นจมูกขนาดความแรง 200 ยูนิตที่เตรียมขึ้นกับยาต้นแบบ กระทำในอาสาสมัครชายสุขภาพดี จำนวน 12 คน โดยใช้แบบแผนการทดลองข้ามสลับชนิดสองทาง อาสาสมัครได้รับยาจำนวน 400 ยูนิต (200 ยูนิตพ่นสองครั้ง) เก็บตัวอย่างพลาสมาและทำการวิเคราะห์โดยใช้เทคนิคทางเรดิโออิมมิวโนโลยี จากการวิเคราะห์ข้อมูลทางสถิติพบว่า ค่าความเข้มข้นของระดับยาสูงสุดในพลาสมา และพื้นที่ใต้เส้นโค้งระหว่างความเข้มข้นของยาในพลาสมากับเวลาของผลิตภัณฑ์ทั้งสองตัวอย่างไม่มีความแตกต่างอย่างมีนัยสำคัญ ($p>0.05$) ค่าร้อยละ 90 ของช่วงความเชื่อมั่นของสัดส่วนของแต่ละพารามิเตอร์ทางเภสัชจลนศาสตร์เทียบกับยาต้นแบบอยู่ภายในช่วงร้อยละ 80 -125 ดังนั้นสามารถสรุปได้ว่ายาพ่นจมูกที่เตรียมขึ้นมีชีวสมมูลกับผลิตภัณฑ์ต้นแบบและมีความเท่าเทียมกันในทางเภสัชกรรม

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BORDEESUDA SUIWONGSA: FORMULATION, STABILITY AND BIOEQUIVALENCE OF SALMON CALCITONIN NASAL SPRAYS. THESIS ADVISOR: ASSOC. PROF. PARKPOOM TENGAMNUAY, Ph. D. 169 pp. ISBN 974-53-2263-6

Salmon calcitonin (CT) is a peptide used in the treatment of osteoporosis and other bone-related disorders. It is currently available in Thailand as an injection and a nasal spray solution, both of which are imported resulting in a high cost of medication and limited usage. The main objective of this study was to develop salmon CT nasal spray preparations that could provide acceptable stability and bioavailability at a more economical cost. Two strengths (100 and 200 IU per actuation) of salmon CT nasal sprays were prepared (two batches each). The formulation was an isotonic solution of synthetic salmon CT with appropriate preservative, buffer and tonicity adjuster. A special spray pump was used that could provide an accurate and reproducible spray volume of 0.09 ml per actuation. The stability studies consisted of real-time testing at 4 °C (recommended storage condition) for 12 months and at 30 °C (accelerated condition) for 4 months. Samples were taken periodically to determine for salmon CT content as well as its degradation product (calcitonin C) and related peptide (N-acetyl-cys^{1}-calcitonin) by HPLC. The percent labeled amount of all four batches was within 90.0 – 115.0 % ranges. The prepared nasal sprays also complied with the tests for calcitonin C and related peptide as well as the clarity, pH, osmolarity, uniformity of mass and sterility tests under both storage conditions. The reproducibility of the pump spray performance was also confirmed based on the results from the leak test, droplet size distribution and spray pattern evaluation. The *in vivo* bioavailability of the test product (200 IU strength) relative to the innovator product was further evaluated in 12 healthy male volunteers. Each subject received a total single dose of 400 IU salmon CT in a two-way crossover study. The plasma concentrations of salmon CT were determined by radioimmunoassay. There were no statistically significant differences in the corresponding pharmacokinetic parameters (AUC and C_{max}) between the two products ($p > 0.05$, ANOVA). The 90% confidence intervals for the ratio of the two parameters (test to innovator) based on the log-transformed data were within the 80.0 –125.0% bioequivalence range. Thus, it can be concluded that the prepared salmon CT nasal spray solutions were both pharmaceutically equivalent and bioequivalent to the innovator product.

Department	Pharmacy	Student's signature Bordeesuda Suiwongsa
Field of study	Pharmacy	Advisor's signature Parkpoom Tengamnuay
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ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

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LIST OF ABBREVIATIONS

%	=	Percent
% L.A.	=	percent labeled amount
°C	=	degree Celsius
µg	=	Microgram
µL	=	Microliter
µm	=	Micrometer
^{125}I	=	Iodine 125
A°	=	Angstrom
ANOVA	=	analysis of variance
AUC	=	area under the plasma concentration – time
B	=	bound radio-labeled antigen
B_o	=	unbound radio-labeled antigen
BMI	=	body mass index
BP	=	British Pharmacopoeia
CDER	=	Center for Drug Evaluation and Research
C.I.	=	confidence interval
C.V.	=	coefficient of variation
cm	=	Centimeter
C_{max}	=	peak plasma concentration
CPM	=	Counts per minute
CT	=	Calcitonin
D_{10}	=	10% of the droplet diameters are smaller than the indicated value
D_{50}	=	50% of the droplet diameters are smaller than the indicated value
D_{90}	=	90% of the droplet diameters are smaller than the indicated value
Da	=	Dalton
EDQM	=	European Directorate for the Quality Medicine
EP	=	European Pharmacopoeia
EPCRS	=	European Pharmacopoeia Control Reference

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FDA	=	Food and drug administration
HPLC	=	high performance liquid chromatography
hr	=	Hour
I.U.	=	international unit
k_e	=	elimination rate constant
kg	=	Kilogram
L	=	Liter
LLOQ	=	lower limit of quantitation
Ln	=	natural logarithms
m	=	Meter
M	=	Molar
Mfg.	=	Manufacturing
m^2	=	square meter
mg	=	Milligram
min	=	Minute
mL	=	Milliter
mm	=	Milliliter
mOsmol	=	Milliosmol
MSE	=	mean square error
MW	=	molecular weight
N	=	Normality
nm	=	Nanometer
No.	=	Number
NSB	=	non - specific binding
pg	=	Pictogram
q.s.	=	quantum sufficiat
r^2	=	coefficient of determination
RIA	=	Radioimmunoassay
rpm	=	revolution per minute
S.D.	=	standard deviation
S.E.	=	standard error
t1/2	=	half life
t_{max}	=	time to peak plasma concentration

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USP	=	United States Pharmacopoeia
UV	=	Ultraviolet
v/v	=	volume by volume
w/v	=	weight by volume
w/w	=	weight by weight
WHO	=	World Health Organization

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