DEVELOPMENT AND CHARACTERIZATION OF TOPICAL ANALGESIC OINTMENT – FROM LABORATORY TO PRODUCTION SCALE

BY

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ABSTRACT

The preparation containing methyl salicylate in an ointment dosage form, is available from various manufacturers throughout the world for the treatment of muscular pain. The limitations of the active ingredients had restricted the use of the ointment only for muscular pain. A new combination of methyl salicylate is proposed with other four active pharmaceutical ingredients (API), formulated as an ointment, that is envisaged to give wholesome benefits to the patients as it contained contemporary APIs, combined with natural products. The APIs were methyl salicylates (MS), camphor and menthol, whereas the natural products were eucalyptus oil (EO) and peppermint oil (PO). Peppermint oil contains menthol as one of the components. A proper scale-up of an ointment is very essential due to high variability of characteristics of semisolid formulation from lab scale as compared to production scale. It was also reported that due to the complex nature of the bases present in the combination of some APIs, product stability may suffer. Hence the objectives of this study were to formulate and characterize the analgesic ointment containing five APIs and identifying its critical quality attributes (CQA), to scale-up the formula from lab size to a pilot size, to develop and validate quantitative analytical method quantifying the presence of the APIs from the dosage form, to identify and optimize critical processing parameters (CPP) for the scale-up batches and finally to conduct the accelerated and real-time stability studies for the scale-up batches. Briefly, the lab scale was formulated from 100g to 5kg batch size, consisting of 25% w/w MS, 5% camphor, 5% menthol, 5% EO and 5% PO, by using the overhead stirrer (100 rpm and 15 mins of mixing time). Different formulations were tested by varying the ratio between the two types of ointment bases, namely, petroleum jelly (PJ) and beeswax (BW). It was found that this lab scale gave the pH between 4.83 to 4.93, the hardness between 31.33 to 35.00g, the viscosity between 558 to 2803 mPa.s and the spreadability from 75 to 1947 mPa.s. The ointment exhibited pseudoplastic behaviour with yield stress and was found to be thixotropic. Three formulations consisted of ratios PJ: BW 10:90 (FI), 30:70 (FII) and 50:50 (FIII) were selected to be scaled-up and characterized. The scaled-up was conducted using a vacuum homogenous mixer and automatic tube filling machine for 35kg batch size. It was identified at this stage that the CPPs of vacuum homogeneous mixer were temperature of mixing and cooling, speed of the agitator and the time of mixing. While, CPPs of automatic tube filling machine were dosage and speed. Critical quality attributes (CQA) were identified as physical characteristic, minimum fill, content based on assay and microbial limit test. The formulations FI to FIII were characterized and exhibited pH range from 4.75 to 4.95, viscosity of 735 to 1670 mPa.s and spreadability of 735 to 1670 mPa.s. The ointment exhibited pseudoplastic behaviour with yield stress and found to be thixotropic. By using gas chromatography coupled with flame iodide detector (GC-FID), analytical method was developed and validated using the scale-up batches and all parameters namely specificity, limit of quantification (LOQ), linearity and range, precision and recovery, and intermediate precision fulfil the specification. After storage up to 6 months, the percentage content of MS, camphor, menthol, EO and PO were 24.6 to 25.9% w/w, 4.6 to 4.8% w/w, 6.8 to 7.5% w/w, 4.95 to 5.2% w/w and 6.85 to 7.1% w/w respectively. Hence, we conclude that a stable ointment consisted of five APIs had been successfully formulated and scaled-up. The predicted shelf life was 2.1 years.

خلاصة البحث

المستحضرات المحتوية على ساليسيلات الميثيل على شكل مرهم متاح من قبل مختلف الشركات المصنعة في جميع أنحاء العالم لعلاج آلام العضلات. قيدت محدودية المكونات النشطة استخدام المراهم فقط لآلام العضلات. اقترحت في هذه الدراسة تركيبات جديدة لساليسيلات الميثيل مع أربعة مكونات صيدلانية نشطة (API)، محضرة كمرهم، والتي من المتوقع أن تعطي فوائد صحية للمرضى لاحتوائها على مكونات صيدلانية نشطة حديثة بالإضافة إلى مركبات طبيعية. المكونات الصيدلانية النشطة كانت ساليسيلات الميثيل، والكافور، والمنثول، والمركبات الطبيعية كانت زيت الكافور، وزيت النعناع. يضمن زيت النعناع المنثول كأحد المكونات. تضخيم انتاج المرهم أمر ضروري بسبب التباين الكبير في خصائص المستحضرات الشبه الصلبة المصنعة مخبريا مقارنة بالإنتاج الصناعي. أكدت التقارير أيضا أنه نظرا للطبيعة المعقدة للقلويات الموجودة في بعض التركيبات للمكونات الصيدلانية النشطة فاستقرار المنتج قد يتأثر. وللك كله هدفت هذه الدراسة لصياغة وتوصيف مرهم مسكن يحتوي على خمسة مكونات صيدلانية نشطة، وتحديد سمات الجودة الهامة الخاصة بها (CQA)، لتضخيم نطاق المستحضر من الحجم المخبري إلى الحجم التجريبي، وللتطوير والتحقق من صحة طريقة للتحليل الكمي لتحليل وجود مكونات صيدلانية نشطة من الشكل الجرعي، ولتحديد وتحسين معايير المعالجة الهامة (CCP) لدفعات التضخيم، وأخيرا هدفت الدراسة لإجراء دراسات الاستقرار المتسارعة والحالية لدفعات التضخيم. باختصار، تم اعداد المستحضر مخبريا من حجم 100 غ إلى 5 كغ لحجم الدفعة والتي تألفت من 25% سائل/سائل ساليسيلات الميثيل، 5% من الكافور، 5% من المنثول، 5% من زيت زيت النعناع، و5% من زيت الأوكالبتوس باستخدام القلاب العلوي (100 دورة في الدقيقة و 15 دقيقة من وقت الخلط). تم اختبار تركيبات مختلفة من خلال تغيير النسبة بين نوعي المادة الأساسية للمرهم، وهما الهلام النفطي وشمع العسل. وجد أن المستحضر المعد مخبريا أعطى مستوى حموضة بين 4.83 إلى 4.93، وصلابة بين 31.33 إلى 35.00 g، ولزوجة بين 558 إلى 2803 مل باسكال، وانتشار من 75 إلى 1947 مل باسكال. أظهر المرهم سلوكا شبه لدني مع تأثير على التحصيل وكان متميعا بالهز. تم اخيار ثلاثة تركيبات بالنسب الآتية: شمع العسل والهلام النفطي 10:90 (FI)، 30:70 (FII) و 50:50 (FIII) لتضخيم انتاجها وبعد ذلك تصنيفها. تم إجراء هذا التضخيم باستخدام خلاط مشفط متجانس وآلة ملء أنبوب تلقائية لدفعة ذات حجم 35 كجم. تم التعرف في هذه المرحلة على أن معايير المعالجة الهامة للخلاط المشفط المتجانس كانت درجة حرارة الخلط والتبريد، وسرعة الهزاز، ومدة الخلط. بينما معايير المعالجة الهامة لكبس أنابيب الملء التلقائية كانت كمية الجرعة والسرعة. تم تحديد سمات الجودة الهامة (CQA) بالخصائص الفيزيائية، والحد الأدنى لملء، المحتوى المعتمد على الفحص واختبار الحد الميكروبي. اتسمت تركيبات FI و FII وأظهرت درجة حموضة تراوحت من 4.75 إلى 4.95، ولزوجة من 735 إلى 1670 مل باسكال، وانتشار من 735 إلى 1670 مل باسكال. أظهر المرهم سلوكا شبه لدني مع تأثير على الإنتاج وكان متميعا بالهز. تم تطوير الطريقة التحليلية والتحقق من صحتها باستخدام الكروماتوغرافيا للغازية مع كاشف لهب الأيوديد (GC-FID) مع دفعات الإنتاج المضخمة وكانت جميع المعلمات وهي التحديد، وحد التحديد الكمي (LOQ)، والخطية والمدى، والدقة والاسترداد، والدقة الوسطية مستوفية للمواصفات. بعد التخزين لمدة وصلت إلى 6 أشهر، كانت نسبة محتوى ساليسيلات الميثيل 24.6 – 25.9% و/و، والكافور 4.6-4.8% و/و، والمنثول 6.8-7.5% و/و، زيت النعناع 4.95-5.2% و/و، وزيت الأوكالبتوس 6.85-7.1% و/و. وختاما استنتج أن مرهما مستقرا يتألف من خمسة مكونات صيدلانية نشطة قد تمت صياغته وتضخيم انتاجه بنجاح، وكانت مدة صلاحيته المتوقعة حوالي 2.1 سنة.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master in Pharmaceutical Sciences (Pharmaceutical Technology).

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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Resolution between an analyte peak and its preceding peak (R_s)	$R_s = \frac{2(T_{R1} - T_{R2})}{(W_1 + W_2)}$
Number of theoretical plate (N)	$\mathrm{N} = 16\left(\frac{T_R}{W}\right)^2$
Retention factor (K´)	$\mathrm{K}' = \frac{(T_R - T_O)}{T_O}$
Tailing factor (T)	$\mathrm{T} = \frac{W_{5.0}}{T_w.2}$
Reproducibility of peak area response (% RSD) where n = 6	$\%\ \mathrm{RSD} = \frac{SD_{area}}{Mean_{area}} X\ 100$
Limit of Quantitation (LOQ)	$LOQ\ = \frac{10\ \sigma}{S}$
% Recovery	$\frac{Amount\ recovered\ (ppm)}{Amount\ added\ (ppm)} \times 100$
First order reaction rate constant (K) per month for each temperature	$\mathrm{Slope} = \frac{-K}{2.303}$
Value of K at 25°C (K_{25}) was extrapolated from the Arrhenius plot and shelf life of the formulation was calculated by substituting the value of K_{25}	$\mathrm{t90} = 0.1054/k25$

LIST OF SYMBOLS

°C	degree Celsius
cm^{-1}	per centimetre
G	Gram
g/mol	Gram per mol
Hz	Hertz
Kg	Kilogram
mg/kg	Milligram per kilogram
mPa.s	milipascal second
s^{-1}	per second

LIST OF ABBREVIATIONS

ALS	Automatic liquid sampler
API	Active pharmaceutical ingredient
ATR-FTIR	Attenuated reflectance infra-red spectroscopy
BP	British Pharmacopoeia
BW	Beeswax
CNS	Central nervous system
CPP	Critical processing parameter
CQA	Critical quality attribute
EO	Eucalyptus oil
EP	European Pharmacopeia
FDA/USFDA	The Food and Drug Administration is a federal agency of the United States Department of Health and Human Services, one of the United States federal executive departments.
FI	Formulation I
FII	Formulation II
FIII	Formulation III
French BIAM	Short form for Banque des Donnes Automatise sur les Medications (word in French). One of the French data bases specialised in the cataloguing of drugs and substances that are created between French universities and the pharmaceutical industries and is used by pharmaceutical laboratories.
GC-FID	Gas chromatography coupled with flame ionization detector
GI	Gastrointestinal
GMP	Good Manufacturing Practice guideline
ICH	International Conference on Harmonization of Technical Requirements for Registration on Pharmaceuticals for Human Use
ICH Q2 (R1)	International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use (ICH) Validation of

	Analytical Procedure
IM	Intramuscular
IR spectra	Infrared spectra
IS	Internal standard
IUPAC	International Union of Pure and Applied Chemistry
IV	Intravenous
LC	Liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantitation
MS	Methyl salicylate
NPRA	National Pharmaceutical Regulatory Agency in Malaysia
NSAIDs	Nonsteroidal anti-inflammatory drug
o/w	Oil-in-water
OTC	Over-the-counter
PEG	Polyethylene glycol polymer
PJ	Petroleum jelly
PO	Peppermint oil
SHS-GC	Static headspace-gas chromatography
SST	System suitability test
TGA	Australian Therapeutic Goods Administration
USA	United States of America
USP	United States Pharmacopoeia
w/o	Water-in-oil
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Pain is a condition that is experienced by human in any stage of life that gives stress to the sensory and an uncomfortable feeling to the body with possible tissue harm. It is not only a sensation, but it is an indication of a disease. According to the World Health Organization (WHO), it is an unpleasant sensory and emotional experience. The pain can be a warning signal, when the body has been damaged. In the lives of human, it plays an important role. The pain serves to protect us from harm and to alert us to diseases or conditions. Even though not all pains need treatment, the pains need to be attended to, in order to treat the actual diseases. Therefore, the management of pain is important to give relieve to the patient.

The pain can be managed by some medication as well as some supportive therapy. These medications can be a modern or a traditional one. The established medication or analgesics include ibuprofen and acetaminophen. The traditional management of pain include application of herbal medicines containing extracts, derived from parts of the plants or other plant materials as active ingredients. The supportive therapies include meditation, heat and cold therapy, massage, and relaxation technique. In addition, topical analgesic is one of the most common ways to get immediate relief from pain. There are a variety of topically available active pharmaceutical ingredients (API) which are formulated as ointment, cream, or spray. They include diclofenac sodium, menthol. and methyl salicylate.

According to Medical Dictionary for the Health Professions and Nursing (2012), the topical analgesic is a compound that can produce analgesia which acts as

pain-relieving drug without causing unconsciousness. One of the characteristics of these topical analgesics is responding to reduction of painful stimulation. Camphor, menthol, methyl salicylate, eucalyptus oil, and peppermint oil are some popularly known and well-established APIs for topical ointment.

The formulation of an ointment varies greatly from lab scale to large scale because of the possible variation process parameter due to the difference in the equipment. During the initial study, lab scale formula was applied to identify the critical quality attributes (CQAs) and during the initial scale-up, critical processing parameters (CPP) were identified. These two factors were essential to ensure appropriate manufacturing scale to be developed and to fulfil regulatory requirement.

1.2 PROBLEM STATEMENT

Methyl salicylates (MS), camphor, menthol, eucalyptus oil (EO) and peppermint oil (PO) are commonly used ingredients in analgesic ointment. These ointments are used separately or combined as two to three ingredients of an ointment. However, till date, to the best of our knowledge, there is no such ointment that combines all these five ingredients together in one dosage form. There are high variability of characteristics of semisolid formulation prepared from lab scale to large scale. Proper scale-up of ointment is very essential in this respect. But there is not much work published on the scale-up considerations of ointment although it is a very common dosage form, perhaps owing to the trade secret. Combination of ingredients might lead to the difficulty in finding suitable formulation to ensure product stability, especially for semisolids due to the complex nature of the bases used. Therefore, imparting stability to the ointment containing large number of APIs is one of the major associated problems.

1.3 RESEARCH OBJECTIVES

The main aim of the research was to formulate and scale up an analgesic ointment containing camphor, menthol, methyl salicylate, eucalyptus oil and peppermint oil to produce it at commercial scale. To achieve this, the research was targeted to the fulfilment of the following objectives:

1- To formulate five APIs combination and characterize the ointment by identifying its CQA.

2- To scale up the ointment while identifying its CPP.

3- To develop and validate the analytical method to quantitatively analyse the five APIs using Gas Chromatography-Flame Ionisation Detector (GC-FID).

4- To carry out the stability study of the developed and scaled-up ointment according to the ICH guidelines (ICH Q1A to ICH Q1F).

CHAPTER TWO

LITERATURE REVIEW

2.1 PAIN

2.1.1 Definition of Pain

Pain often refers to unpleasantness and discomfort or any negative emotion and negative effect of a sensory experience. It can range from mild to extreme level. Pain can probably be resulting from an actual tissue damage to body or can occur even in the absence of any physical harm. Pain can be influenced by a various source variability that comes from nurture (environment) and nature (genes). Environmental factors include psychological and personality related factors such as previous pain experience, emotionality, anxiety, and fear. Molecular genetic of pain includes the identified genetic risk factors contributing to the pain in human (Atlas & Wager, 2012; Belfer, 2013; National Institutes of Health [NIH], 2011).

2.1.2 Types of pain

One of the common classifications of pain is based on duration. It can distinguish pain into categories called acute and chronic pains. Acute pain lasts for a short time. The time frame is less than three months and maybe as brief as seconds (Radnovich et al., 2014). These include post-operative pain which requires only short-term care. Chronic pain, in contrast, lasts beyond the healing of an injury and can continue for a period of more than three months. It lasts longer due to the nature and symptoms of disease that requires multi-therapeutic activities and long term care management (Koneti & Jones, 2013; Radnovich et al., 2014; Swieboda, Filip, Prystupa, & Drozd, 2013).

2.1.3 Management of pain

Pain needs to be controlled as it can affect the quality of life. If pain is not treated, it can affect the individual's sleep and decrease appetite. The comprehensive pain managements generally include pharmacologic intervention and non-pharmacologic intervention. Pharmacologic intervention is also known as pharmacologic therapy which is a type of medical care that involves medication either alone or in combination with other types of therapy. There is an alternative way to control pain without the use of medicines, which is known as non-pharmacologic therapy or non-pharmacologic intervention. This may include changes in life-style such as diet, exercise and control of smoking and alcohol consumption.

Pharmacologic therapies involve several classes of drug. The three categories that treat pain are acetaminophen, nonsteroidal anti-inflammatory drugs (NSAIDs), and opioids. For neurological pain, pharmacological intervention includes anticonvulsant and gabapentin as recommended by WHO (Keskinbora, Pekel, & Aydinli, 2007). But for severe neuropathic pain, local anaesthetics such as transdermal lignocaine and oral lignocaine analogue, Mexiletine is given to relieve the pain (Clancy, 2012). In addition, adjuvants may also be prescribed, those include muscle relaxants and anticonvulsants (Nalamachu, 2013). Breast cancer patients receiving chemotherapy usually have problems with cognitive alterations. These patients receive pharmacologic interventions including psychostimulants, epoetin alfa, and ginkgo biloba (Chan, McCarthy, Devenish, Sullivan, & Chan, 2015).

Generally, the non-pharmacological treatment that is used to control the pain includes application of heat, ice, massage, and physical therapies. In addition, aroma therapy, guided imagery, laughter, music, self-hypnosis, and acupuncture are commonly used. The types of non-pharmacological treatment methods, used to relieve