ABSTRACT

The present invention relates to a novel, cost-effective and simple process for commercial scale production of methoxsalen which obviates the use of column chromatography for purification of dealkylated mass obtained from *Ammi majis* plant extract to obtain purified dealkylated mass enriched with methoxsalen and xanthotoxol with low impurity, which is suitable for methylation to obtain a pharmaceutical grade methoxsalen.

10 JUN 2013

1401/22330/2014. MUM

We claim:

1. A process for purification of dealkylated mass obtained from *Ammi majus* extract, which comprises:
   a) multiple extraction of the crude dealkylated mass with toluene at a given temperature with specified volumes for about one hour followed by collection of residue by filtration;
   b) further extraction of the residue with an alcohol at a specified volume and temperature for about half an hour;
   c) filtration of residue;
   d) washing with alcohol and
   e) drying the obtained residue.

2. The process according to claim 1, wherein the alcohol is selected from the group consisting of methanol, ethanol, propanol, or the mixture thereof.

3. The process according to claim 1, wherein the alcohol is methanol.

4. The process according to claim 1, wherein the ratio of Toluene used in the extraction ranges from about 1.2 times to 0.2 times (w/v) with respect to the starting crude dealkylated mass.

5. The process according to claim 1, wherein the toluene extraction is carried out temperature in the range of 50^0C-65^0C.

6. The process according to claim 1, wherein the ratio of alcohol used in the extraction ranges from about 0.4 times to 0.1 times (w/v) with respect to the starting crude dealkylated mass.

7. The process according to claim 1, wherein the alcohol extraction is carried out temperature in the range of 25^0C-35^0C.

8. The process according to claim 1, wherein the dealkylated mass obtained consists of Methoxsalen 20%-50%.and Xanthotoxol 40%-80%.as active ingredients.

-9 JUN 2014

9. The process according to claim 1, wherein the total impurities present in purified dealkylated mass obtained is <1%.

10. The process of claim 1, wherein the obtained purified dealkylated mass is methylated to get pharmaceutical grade of methoxsalen.

Dated this 7th day of June 2014

Dr. RAMESH KUMAR
Assistant General Manager-IPM
Sequent Scientific Ltd.

- 9 JUN 2014

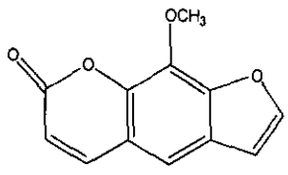
FIELD OF INVENTION

The present invention relates to a novel, cost-effective process for preparation of Methoxsalen from an extract of *Ammi majus*. Specifically, it relates to a process for the purification of dealkylated mass extracted from seeds of *Ammi majus* plant for the preparation of pharmaceutical grade of Methoxsalen.

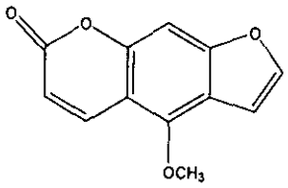
BACKGROUND OF THE INVENTION

Biological Source of Methoxsalen is a naturally occurring analogue of Psoralen, found in various species of *Rutaceae*, *Leguminosae*, and *Umbelliferae*. It is being extracted commonly from the seeds of *Ammi majus* plant also called as *Bishop's weed.*

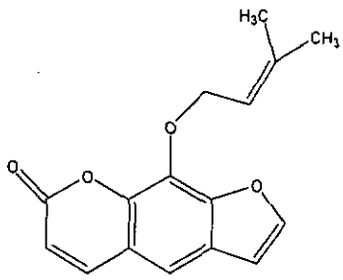
Methoxsalen, also known as xanthotoxin or 8-Methoxypsoralen, is represented by formula I. It is a drug used to treat psoriasis, eczema, vitiligo, and some cutaneous lymphomas in conjunction with exposing the skin to sunlight. The seeds of *Ammi majus* plant are rich in other furanocoumarins like Bergapten, Imperatorin, Isopimpinellin, Psoralen, Xanthotol etc. represented by following formulas.



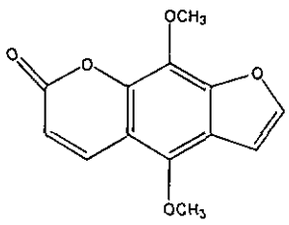
I



Bergapten

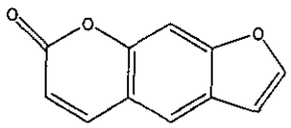


Imperatorin

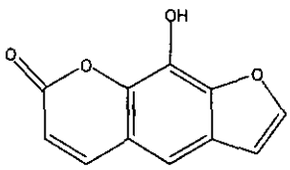


Isopimpinellin

- 9 JUN 2014



Psoralen



Xanthotoxol

The furanocoumarins are commonly extracted from *Ammi majus* seeds using suitable solvents like non polar hydrocarbons, alcohols or ethyl acetate to yield a crude residue containing the above specified furanocoumarins and esters of linoleic, Oleic, palmetic acids and other fixed oils.

The Furanocoumarin fraction contains methoxsalen, imperatorin, isopimpinellin and bergapten as major compounds. It is known that imperetorin on acid hydrolysis gives the hydroxyl derivative of methoxsalen which is called as Xanthotoxol.

To prepare Methoxsalen from crude extract in commercial scale, the dealkylated mass of the crude extract is selected as the starting material which is prepared by subjecting the crude extract to acid hydrolysis in alcohol medium.

A typical furanocoumarin composition of dealkyalted crude from *Ammi majus* extract is as below:

Compounds	Content (w/w)
Methoxsalen	25% approx.
Xanthotoxol	15% approx..
Isopimpinellin	10% Approx.
Bergapten	5% approx.
Imperetorin	0.5% approx.
Psoralen	0.5% approx.

Commercial preparation of pharmaceutical grade of Methoxsalen from the crude extract of *Ammi majus* is a challenge considering the stringent impurity specifications. As per ICH guideline for the Active Pharmaceutical ingredients, the other furanocoumarins need to be reduced to <0.2% individually.

Therefore as a standard process, dealkyalted crude of *Ammi majus* extract is first purified to remove the impurities and enriching it with methoxsalen and

= 9 JUN 2014

xanthotoxol. The purified dealkylated mass obtained in this method is methylated by known methods to obtain pharmaceutical grade of methoxsalen.

Considering the complex composition of furanocoumiarins in the crude and dealkylated extract of *Ammi majus* seed, column chromatography is considered as the ideal process to separate these impurities. The other common technique is solvent purification followed by crystallisation. Following are the observation made while applying these concepts.

A. Purification by column chromatography

The crude dealkylated mass is subjected to chromatographic separation using silica gel as the stationary adsorbent and combination of organic solvents at different concentration as mobile solvent. The organic solvent includes but not limited to hexane, ether, alcohols, ethyl acetate and chlorinated solvents. The major disadvantage of the process is that methoxsalen, bergapten and isopimpinellin co-elute in most of the mobile system. A further separation of impurities is found to be difficult. Other disadvantages include the requirement of multiple adsorption and relatively very high cost in commercialization due to large volumes of solvent and effluents.

B. Solvent purification

Alternatively various solvent purification methods using multiple solvents have been employed individually and in combination for the purification of dealkylated mass. Crystallization was also employed to achieve the desired output.

The results of these experiments were not satisfactory as the purified product had either very low recovery or high content of impurities or both, thus making it unsuitable for commercial production of pharmaceutical grade of methoxsalen.

Thus there is a need to develop a process for purification of dealkylated mass obtained from Ammi majus, which is cost effective, has high recovery and high purity.

SUMMARY OF INVENTION

The present inventors have developed a novel process for purification of dealkylated mass obtained from *Ammi majus* extract. The process comprises:

- 9 JUN 2014

a) multiple extraction of the crude dealkylated mass with toluene at a given temperature with specified volumes for about one hour followed by collection of residue by filtration;
b) further extraction of the residue with alcohol at a specified volume and temperature for about half an hour;
c) filtration of residue;
d) washing with alcohol and
e) drying the obtained residue.

Thus obtained pure dealkylated mass is subjected to methylation by known methods to prepare pharmaceutical grade of methoxsalen.

DETAIL DESCRIPTION OF INVENTION

In an embodiment of the invention, the purification of dealkylated mass obtained from *Ammi majus* extract is done by using toluene and an alcohol selected from the group consisting of methanol, ethanol, propanol *etc.* preferably methanol to reduce the impurities and enrich it with methoxsalen and xanthotoxol. The purified extract is suitable for the preparation of methoxsalen confirming the active pharmaceutical ingredient grade specification. The solvent volumes, extraction temperature and extraction time are critical to achieve the desired purified product.

In another embodiment of the invention, the process involves extraction of the crude dealkylated mass with toluene four times at specified volumes and temperature for about one hour.

In another embodiment of the invention, ratio of dealkylated mass to the volumes of toluene for all the four extractions are at the ratio (w/v) of about 1:1.2, about 1:1, about 1:0.6 & about 1:06 respectively and the extraction temperature is at about 50 to 65°C, preferably at 60±2°C.

The ratio of dealkylated mass to the volume of alcohol used is about 1:0.4 (w/v) for extraction at about 25°C to 35°C, preferably at 28±2°C and about 1: 0.2 w/v for rinsing at 0-10^{0}C.

In another embodiment of the invention, the mixture is filtered and the residue is taken for further extraction after each extraction. The toluene residue after final

9 JUN 2014

extraction is further extracted with an alcohol selected from the group consisting of methanol, ethanol, propanol *etc.* preferably methanol at a specified volume and temperature for about half an hour. The mixture is again filtered and the residue is rinsed with alcohol. The wet residue is dried under vacuum to remove the residual toluene, alcohol and water. The dried material is powdered and subjected to methylation by known methods or by a process illustrated in example 2 to prepare pharmaceutical grade of methoxsalen.

In another embodiment of the invention, it is observed that at lower solvent ratio and temperature, the impurities may not be reduced to an acceptable level of purity of dealkylated mass for preparation of pharmaceutical grade of methoxsalen. Whereas, a higher solvent volume and temperature lead to poor recovery of Methoxsalen and Xanthotoxol.

In another embodiment of the invention, the process of the present invention has high recovery of Xanthotoxol and Methoxsalen which makes it commercially viable involving only simple extractions steps. A relatively non-polar solvent like toluene removes the non-polar impurities like isopimpinellin, bergapten, fatty acid esters etc. very selectively at particular solvent ratio and temperature. The residual polar impurities are removed by an alcohol and thus making the product enriched with only Methoxsalen & Xanthotoxol suitable for the preparation of Methoxsalen acceptable to pharmaceutical standards.

It should be noted that the 10-15% variation in the ratio of specified solvents and temperature will still lead to dealkylated mass with acceptable level of purity, which will fall within the scope of this invention.

The process of the present invention can be further illustrated by the below examples.

Example 1: Purification of dealkylated mass

Crude de-alkylated mass (5.0Kg) and toluene (6 L) were heated to 60 ± 2^{0}C on a water bath under stirring and maintain the temperature of the extraction for 1 hour. The mixture was filtered under vacuum. The residue was again extracted with

-9 JUN 2014

toluene (5 L) at 60±2°C for 1 hour. The mixture was filtered under vacuum. The residue was again extracted twice with toluene (3 L) at 60±2°C for 1 hour. The residue was further extracted with methanol (2L) at 28±2°C for 30 minutes. The mixture was filtered and the residue was rinsed with chilled methanol and dried under vacuum. The dry product was powdered and subjected to HPLC analysis.

Product obtained from the process has the following composition

Methoxsalen	Xanthotoxol	Bergapten, Isopimpinellin & related impurities
20%-50%	40%-80%	<1%

Example 2: Preparation of Methoxsalen

Dimethyl sulphate (0.82kg) was added to a suspension of purified dealkylated mass (1.33kg) and anhydrous potassium carbonate(0.9kg) in methanol(6.65L) at 25-30°C under stirring and heated to 45-50°C for 3hrs. After completion of the reaction, methanol was distilled out from the mass under vacuum to a residue and water (6.65L) was added to precipitate crude material. The material was filtered and dried at 50-60°C under vacuum.

The crude material was dissolved in methanol (53.2L) at reflux and treated with activated charcoal (0.0665kg). The obtained clear filtrate was concentrated, crystallised. The crystals were filtered and dried at 50-60°C under vacuum till moisture was 0.3%. Yield 1.0kg.

The HPLC analysis of the obtained methoxsalen is as below:

Purity more than 99.5%

Bergapten content: less than 0.1%

Isopimpinellin content: less than 0.1%

Any other impurity: less than 0.1%

- 9 JUN 2014

1401/22330/2014. MUM

We claim:

1. A process for purification of dealkylated mass obtained from *Ammi majus* extract, which comprises:
   a) multiple extraction of the crude dealkylated mass with toluene at a given temperature with specified volumes for about one hour followed by collection of residue by filtration;
   b) further extraction of the residue with an alcohol at a specified volume and temperature for about half an hour;
   c) filtration of residue;
   d) washing with alcohol and
   e) drying the obtained residue.

2. The process according to claim 1, wherein the alcohol is selected from the group consisting of methanol, ethanol, propanol, or the mixture thereof.

3. The process according to claim 1, wherein the alcohol is methanol.

4. The process according to claim 1, wherein the ratio of Toluene used in the extraction ranges from about 1.2 times to 0.2 times (w/v) with respect to the starting crude dealkylated mass.

5. The process according to claim 1, wherein the toluene extraction is carried out temperature in the range of 50^0C-65^0C.

6. The process according to claim 1, wherein the ratio of alcohol used in the extraction ranges from about 0.4 times to 0.1 times (w/v) with respect to the starting crude dealkylated mass.

7. The process according to claim 1, wherein the alcohol extraction is carried out temperature in the range of 25^0C-35^0C.

8. The process according to claim 1, wherein the dealkylated mass obtained consists of Methoxsalen 20%-50%.and Xanthotoxol 40%-80%.as active ingredients.

-9 JUN 2014

9. The process according to claim 1, wherein the total impurities present in purified dealkylated mass obtained is <1%.

10. The process of claim 1, wherein the obtained purified dealkylated mass is methylated to get pharmaceutical grade of methoxsalen.

Dated this 7th day of June 2014

Dr. RAMESH KUMAR
Assistant General Manager-IPM
Sequent Scientific Ltd.

- 9 JUN 2014