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(54) Benevnelse **METHOD FOR PREPARING DIOSMIN**

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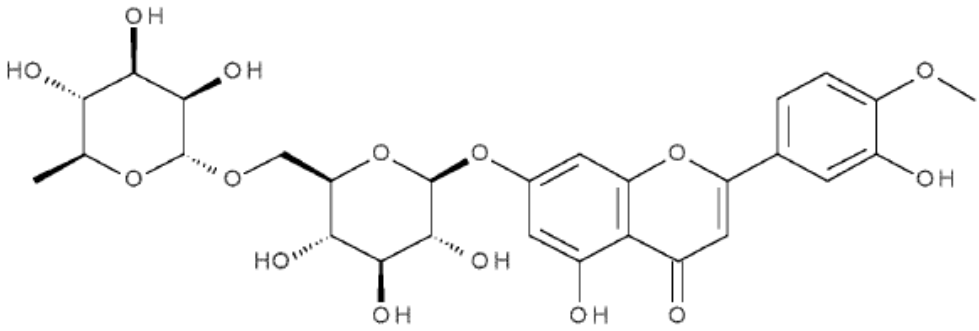
Description

The present invention relates to a method for the preparation of diosmin.

Diosmin is the compound with the formula (I):

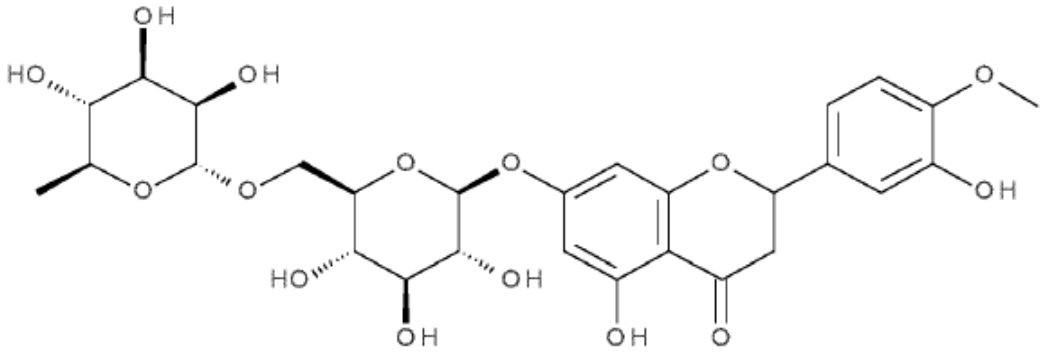


(I)

Diosmin is used in the treatment of venous diseases, such as chronic venous insufficiency or haemorrhoidal diseases.

Diosmin is also the major component of micronised purified flavonoid fraction, or MPFF (Daflon®).

Diosmin is synthesised by oxidation of hesperidin. Hesperidin is the compound with the formula (II):



(II)

Hesperidin is obtained from natural substances (oranges). The diversity of oranges used leads to hesperidins of unequal purity, containing other flavonoids with variable contents. In particular, the hesperidin may contain up to 4% of isonaringin, which is converted to isorhoifolin by oxidation.

Therefore, diosmin generally contains other flavonoids, some of which originate from the oxidation of the flavonoids present in the initial hesperidin, and others are reaction by-products.

Given the pharmaceutical interest of diosmin, it is essential to obtain same with an excellent yield and the required purity, irrespective of the source of hesperidin.

The specifications imposed by the European Pharmacopoeia are the following:

Substances	Diosmin specifications (European Pharmacopoeia)
Diosmin	90.0 to 102.0%
Hesperidin	< 4.0%
Diosmetin	< 2.0%
Isorhoifolin	< 3.0%
Linarin	< 3.0%
6-Iododiosmin	< 0.6%

In particular, it is essential that the diosmin obtained contain less than 0.6% of 6-iododiosmin and less than 3.0% of isorhoifolin.

Methods for the preparation of diosmin from hesperidin have been described in the literature.

FR2311028 describes a method for obtaining diosmin by acetylation of hesperidin followed by oxidation of the acetylated hesperidin by bromination, basic hydrolysis and isolation. The crude diosmin thus obtained is purified by a retreatment step using pyridine.

Such method is not ideal, since the yield is only 65%. Furthermore, the method uses pyridine, a class 3 carcinogenic solvent like in FR2782518 which describes a method of fabrication of diosmin starting from hesperidin by reaction with iodine and pyridine.

Patent application WO2016/124585 has the advantage of not using organic solvents such as pyridine. However, the method that is described therein does not serve to obtain diosmin with the required purity when the hesperidin used contains a large amount of isonaringin.

One of the problems for the present invention was to minimise the content of 6-iododiosmin in the diosmin obtained, while dispensing with the use of class 3 solvents such as pyridine.

Another problem for the present invention was to minimise the content of isorhoifolin in the diosmin obtained, while dispensing with the use of class 3 solvents such as pyridine, while starting from a hesperidin containing up to 4% of isonaringin.

More specifically, the present invention relates to a method for the preparation of diosmin by

a) acetylation of hesperidin,
b) oxidation of the acetylated hesperidin to acetylated diosmin by an iodine donor, at a temperature of from 90 to 120°C,
c) heating of the acetylated diosmin, in an autoclave at a pressure of from 5 to 8 bar, under reflux of an alcohol such as methanol, ethanol or isopropanol, in the presence of a base chosen from sodium acetate or potassium acetate, sodium hydroxide, potassium hydroxide or lithium hydroxide, potassium carbonate, sodium methanolate or sodium ethanolate, alone or in a mixture with another of said bases, then
d) deprotection of the acetylated diosmin to diosmin by heating in the presence of a base chosen from sodium hydroxide, potassium hydroxide or lithium hydroxide, potassium carbonate, sodium methanolate or sodium ethanolate, alone or in a mixture with sodium acetate or potassium acetate,
e) purification by base/acid treatment.

According to one embodiment of the present invention, the diosmin obtained contains other flavonoids such as hesperidin, isorhoifolin, linarin or diosmetin.

According to one embodiment of the present invention, the acetylation step (a) is carried out by reacting hesperidin with acetic anhydride and potassium or sodium acetate.

The acetylation reaction is preferentially carried out at a temperature between 40°C and 135°C.

The amount of acetic anhydride is preferentially between 8 and 10 molar equivalents relative to the hesperidin used.

The iodine donor used in the oxidation step (b) is preferentially chosen from NaI/H_2O_2, KI/H_2O_2, TBAI/H_2O_2 and NaI/I_2 (pref. 9/1) /H_2O_2.

The amount of NaI is preferentially from 0.05 to 0.20 molar equivalent relative to the hesperidin used.

The amount of hydrogen peroxide is preferentially from 1.0 to 1.2 molar equivalents relative to the hesperidin used.

According to one embodiment of the present invention, the acetylated diosmin obtained at the end of the oxidation step (b) is isolated, preferentially by precipitation in water before being used in step c).

According to one embodiment of the present invention, the base used for step c) is an aqueous solution of sodium hydroxide or potassium hydroxide, an aqueous solution of sodium acetate or potassium acetate, or a mixture of sodium hydroxide or potassium hydroxide and sodium acetate or potassium acetate in aqueous solution.

The sodium acetate or potassium acetate used for step c) can be generated *in situ* by neutralisation of the residual acetic acid present in the acetylated diosmin with sodium hydroxide or potassium hydroxide.

The amount of base used in step (c) is preferentially between 0.5 and 2.5 molar equivalents relative to the hesperidin used.

According to one embodiment of the present invention, the base added in the deacetylation step (d) is sodium hydroxide or potassium hydroxide.

The amount of base added in the deacetylation step (d) is preferentially between 2 and 4.5 molar equivalents relative to the hesperidin used.

According to one embodiment of the present invention, the base/acid treatment (step e) is carried out by dissolving into solution in water in the presence of a base such as sodium hydroxide, then precipitation by salification with an acid, such as sulfuric acid.

The following examples illustrate the invention.

Abbreviations:

mol eq	molar equivalents (relative to the hesperidin)
HPLC	High Performance Liquid Chromatography
w/w	ratio expressed as weight/weight
TBAI	tetra-n-butylammonium iodide
vol	volume equivalents (relative to the hesperidin)

EXAMPLE 1: Diosmin

Step A: Acetylated diosmin

Feed into a reactor between 20-25°C, potassium acetate (98.6 mmol) and acetic anhydride (2996.2 mmol).

Heat the suspension, while stirring, to 40°C then introduce hesperidin (2 × 163.8 mmol; HPLC titer: 91.3%, isonaringin 3.8%). Continue stirring while heating at 40°C, then heat to 132°C in 45 min while stirring. The mixture changes to a clear solution at the end of the heating. Stir the solution for 60 min at 132°C then cool to 105°C.

Feed in aqueous sodium iodide solution (33 mmol in 20 g of water). At 105°C, pour in 35% hydrogen peroxide (341.5 mmol) stabilised with 0.1% sulfuric acid.

Stir for 30 min at 105°C then cool to 100°C while stirring and precipitate in a beaker containing water (approx. 7 vol), under mechanical stirring at 20-40°C.

After 30 min of stirring between 20-40°C, filter *in vacuo*, wash the cake with water (9 vol; then 2 × 2 vol). Expurgate for 16 h *in vacuo* between 20-25°C.

Step B: Diosmin

Feed into an autoclave the acetylated diosmin obtained in step A and methanol (3.5 vol). Begin stirring then heat under reflux at a pressure of 5 bar. After 15 min under reflux, feed in sodium hydroxide as a 30% aqueous solution (1.2 mol eq). Heat under reflux for 30 min then cool to 50°C at normal pressure and feed in sodium hydroxide as a 30% aqueous solution (2.4 mol eq). After 2 h at 50°C, cool to 20°C then filter and wash the cake with methanol (2 × 3 vol).

Dissolve the crude diosmin in 2.5 mol eq of sodium hydroxide and water (2.5 vol) at 20°C.

Add sulfuric acid to adjust the pH to between 2 and 4. Maintain for 30 min at 20°C, filter, wash twice with water (2 × 5 vol) and dry.

Yield starting from hesperidin: 83.8%

Purity (HPLC): 90.6%

Content of 6-iododiosmin: 0.3%

Content of isorhoifolin: 2.0%.

EXAMPLE 2: Diosmin

Step A: Acetylated diosmin

Feed into a reactor between 20-25°C, potassium acetate (207.1 mmol) and acetic anhydride (6291.9 mmol).

Heat the suspension, while stirring, to 100°C then introduce hesperidin (5 × 137.6 mmol; HPLC titer: 91.7% and isonaringin 3.6%). Continue stirring while heating at 100°C, then heat to 132°C in 15 min while stirring. The mixture changes to a clear solution at the end of the heating. Stir the solution for 120 min at 132°C then cool to 105°C.

Introduce aqueous sodium iodide solution (68.8 mmol in 40 g of water). At 105°C, pour in 35% hydrogen peroxide (717.1 mmol) stabilised with 0.1% sulfuric acid.

Stir for 30 min at 105°C then cool to 100°C while stirring and precipitate in a beaker containing water (approx. 7 vol), with mechanical stirring at 20-40°C.

After 30 min of stirring between 20-40°C, filter *in vacuo*, wash the cake with water (9 vol; then 2 × 2 vol). Expurgate for 16 h *in vacuo* between 20-25°C.

Step B: Diosmin

Feed into an autoclave, the acetylated diosmin obtained in step A and methanol (3.5 vol). Place under stirring then heat under reflux at a pressure of 5 bar. After 15 min under reflux, introduce sodium hydroxide as a 30% aqueous solution (1.55 mol eq). Heat under reflux for 30 min then cool to 50°C at normal pressure and introduce sodium hydroxide as a 30% aqueous solution (2.4 mol eq). After 2 h at 50°C, cool to 20°C then filter and wash the cake with methanol (2 × 3 vol).

Dissolve the crude diosmin in 2.5 mol eq of sodium hydroxide and water (2.5 vol) at 20°C.

Add sulfuric acid to adjust the pH to between 2 and 4. Maintain at 20°C for 30 min, filter, wash twice with water (2 × 5 vol) and dry.

Yield starting from hesperidin: 81.2%

Purity (HPLC): 90.4%

Content of 6-iododiosmin: 0.29%

Content of isorhoifolin: 2.2%.

EXAMPLE 3: Diosmin

Feed into an autoclave, the acetylated diosmin obtained in step A of Example 1 and methanol (3.5 vol), add 2 mol eq of an aqueous solution of potassium acetate then heat under reflux at a pressure of from 7 to 8 bar. Next, cool to 50°C and feed in an aqueous solution of potassium hydroxide (4.2 mol eq). After contact at 50°C, cool to 20°C, then filter and wash with methanol (2 × 1.5 vol).

Dissolve the crude diosmin in 2.5 mol eq of sodium hydroxide and water (2.5 vol) at 20°C.

Add sulfuric acid to adjust the pH to between 2 and 4. Maintain at 20°C for 30 min, then filter, wash twice with water (2 × 5 vol) and dry.

Yield starting from hesperidin: 87.7%

Purity (HPLC): 90.1%

Content of 6-iododiosmin: not detected (< 0.10%)

EXAMPLE 4: Acetylated diosmin with different iodine donors

Into a 25-ml three-necked flask equipped with an ovoid stirrer and a syringe driver, feed 10 g of hesperidin, 0.5 g of potassium acetate and 14 ml/15.6 g of acetic anhydride. Gradually bring the temperature to 132°C and leave at 130°C for 1 h.

Cool to approximately 90°C then feed in 0.322 g of sodium iodide or the equivalent iodine donor and 2.258 g of water. Heat to 105°C then add 35% hydrogen peroxide (1.1835 ml/1.645 g) and 5.161 g of water.

Test	3a	3b	3c	3d
Hesperidin	10 g	2 g	2 g	2 g
Acetic anhydride	9.146 mol eq	9.146 mol eq	9.146 mol eq	9.146 mol eq
Potassium acetate	0.327 mol eq	0.327 mol eq	0.327 mol eq	0.327 mol eq
35% H_2O_2	1.036 mol eq	1.033 mol eq	1.033 mol eq	1.033 mol eq
H_2SO_4	/	0.00109 mol eq	0.00109 mol eq	0.00109 mol eq

Test	3a	3b	3c	3d
Iodine donor	NaI 0.133 mol eq	KI 0.133 mol eq	TBAI 0.133 mol eq	NaI / I_2 9/1 0.120/0.013 mol eq
Acetylated diosmin	94%	> 97%	> 98%	> 97%

EXAMPLE 5: Diosmin with different bases

Feed into an autoclave the acetylated diosmin obtained in step A of Example 2 and methanol (3.5 vol). Begin stirring then heat under reflux at a pressure of 7 bar. After 15 min under reflux, feed in the base as a 30% aqueous solution (1.2 mol eq). Heat under reflux for 30 min then cool to 50°C at normal pressure and introduce the base as a 30% aqueous solution (2.4 mol eq). After 2 h at 50°C, cool to 20°C then filter and wash the cake with methanol (2 × 3 vol).

Test	4a	4b	4c	4d	4e
Base	CH_3ONa	NaOH	LiOH	KOH	K_2CO_3
Yield / Hesperidin used	84%	84%	85%	84%	80%
Diosmin	89.8%	90.9%	90.4%	90.8%	92.4%
Isorhoifolin	2.6%	2.0%	2.1%	2.0%	2.1%
6-Iododiosmin	0.45%	0.46%	0.42%	0.62%	0.54%

EXAMPLE 6 (comparative): Reproduction of the method of WO 2016/124585

40 g of acetic anhydride, 0.75 g of potassium acetate and 30 g of hesperidin (purity 91.3%; isonaringin 3.8%) are fed into a reactor. The reaction medium is then heated to 115-120°C, maintaining said temperature for one hour approximately, and then the medium is cooled to 60-70°C.

A solution of sodium iodide (0.9 g) in water (6 ml) is added, and the reaction medium is heated to reflux. A solution of 35 ml of 5.4% (by weight) hydrogen peroxide stabilised with sulfuric acid is then added to the reaction medium, while maintaining the reflux. Then, the reaction medium is next cooled to 40-50°C and

potassium hydroxide (10 g) is added to the reaction mixture; the pH is then 4. The mixture is then heated at 115-120°C for 3 hours, and then cooled to 30°C.

The reaction mixture is added to a reactor containing a 2N aqueous sodium hydroxide solution (300 ml). After 1 h and 30 min, sulfuric acid is added until the pH reaches 7.5. The precipitate is then filtered and washed with water, to obtain wet crude diosmin.

The crude diosmin thus obtained is crystallised by dissolving same in an aqueous solution of sodium hydroxide, then by acidifying same with sulfuric acid until the product precipitates.

The solid is filtered, washed with water and dried.

Analysis (HPLC):

Substances	Percentage in the product of Example 5	Diosmin specifications (European Pharmacopoeia)
Diosmin	87.1%	90.0 to 102.0%
Isorhoifolin	3.6%	< 3.0%
6-Iododiosmin	0.99%	< 0.6%

Patentkrav

1. Fremgangsmåte for fremstilling av diosmin ved

a) acetylering av hesperidin,

b) oksidering av acetylert hesperidin til acetylert diosmin via en joddonor, ved en temperatur på 90 til 120 °C,

c) oppvarming av acetylert diosmin i en autoklav ved et trykk på 5 til 8 bar, ved tilbakeflyt av en alkohol i nærvær av en base valgt blant natrium- eller kaliumacetat, natrium-, kalium- eller litiumhydroksid, kaliumkarbonat, natriummetoksid eller natriumetoksid, alene eller i blanding med en annen av disse basene, og deretter

d) avbeskyttelse av acetylert diosmin til diosmin ved oppvarming i nærvær av en base valgt blant natrium-, kalium- eller litiumhydroksid, kaliumkarbonat, natriummetoksid eller natriumetoksid, alene eller i blanding med natrium- eller kaliumacetat,

e) rensing med base/syrebehandling.

2. Fremgangsmåte ifølge krav 1, der det oppnådde diosminet inneholder andre flavonoider.

3. Fremgangsmåte ifølge et hvilket som helst av kravene 1 eller 2, der det oppnådde diosminet inneholder mindre enn 0,6 % 6-joddiosmin og mindre enn 3,0 % isorhoifolin.

4. Fremgangsmåte ifølge et hvilket som helst av kravene 1 til 3, der mengden av eddiksyreanhydrid er mellom 8 og 10 molekvivalenter i forhold til det anvendte hesperidinet.

5. Fremgangsmåte ifølge et hvilket som helst av kravene 1 til 4, der acetyleringsreaksjonen (a) utføres ved en temperatur mellom 40 °C og 135 °C.

6. Fremgangsmåte ifølge et hvilket som helst av kravene 1 til 5, der joddonoren er valgt blant NaI/H_2O_2, KI/H_2O_2, TBAI/H_2O_2 og NaI/I_2 /H_2O_2.

7. Fremgangsmåte ifølge krav 6, der joddonoren er Nal i en mengde på 0,05 til 0,2 molekvivalenter i forhold til det anvendte hesperidinet.

8. Fremgangsmåte ifølge et hvilket som helst av kravene 1 til 7, der mengden av hydrogenperoksid er 1,0 til 1,2 molekvivalenter i forhold til det anvendte hesperidinet.

9. Fremgangsmåte ifølge et hvilket som helst av kravene 1 til 8, der det acetylerte diosminet som er oppnådd etter oksideringstrinnet (b), isoleres ved utfelling i vann før det anvendes i trinn c).

10. Fremgangsmåte ifølge et hvilket som helst av kravene 1 til 9, der basen som brukes for trinn c), er natrium- eller kaliumhydroksid i vannholdig løsning, natrium- eller kaliumacetat i vannholdig løsning eller en blanding av natrium- eller kaliumhydroksid og natrium- eller kaliumacetat i vannholdig løsning.

11. Fremgangsmåte ifølge et hvilket som helst av kravene 1 til 10, der alkoholen som brukes for trinn c), er metanol, etanol eller isopropanol.

12. Fremgangsmåte ifølge et hvilket som helst av kravene 1 til 11, der basemengden som brukes i trinn (c), er mellom 0,5 og 2,5 molekvivalenter i forhold til det anvendte hesperidinet.

13. Fremgangsmåte ifølge et hvilket som helst av kravene 1 til 12, der basemengden som tilsettes i deacetyleringstrinnet (d) er mellom 2 og 4,5 molekvivalenter i forhold til det anvendte hesperidinet.