

Purification of Anthracycline GlycosidesDESCRIPTION

The invention relates to a process for the purification of anthracycline glycosides by selective adsorption on resins.

Raw anthracycline glycosides, whether prepared by fermentation or by synthetic processes, contain particularly high amounts of organic and inorganic impurities, averaging from 12% to 25% impurities. Conventional purification methods, comprising extraction of the raw product with chlorinated solvents and subsequent washings with buffers lead to a final product in which the impurity percentage, although considerably lowered, is in the range of from 4.5 to 5.0%. Bearing in mind the clinical use of the anthracycline glycosides as antitumour agents, and the side problems of dosage and toxicity, the optimum elimination of impurities, generally highly toxic, is clearly desirable.

The invention provides a process for the purification of a raw anthracycline glycoside, obtained by fermentation or synthetically, the process comprising (i) adsorbing an aqueous solution having a pH of from 3 to 5 of the raw anthracycline glycoside on a polymeric or ion exchange adsorbing resin or on carboxy-methylcellulose and (ii) de-adsorbing the purified anthracycline glycoside by elution with water or with slightly acidic water or with a mixture of water and a polar solvent or with a mixture of slightly acidic water and a polar solvent.

The adsorbing resin may be contained in a suitable vessel. Generally, a tower or column filled

with the resin in particulate form, e.g. granules or beads, is used. Depending on the type of impurities contained in the raw product, the eluate provided by step (ii) may be submitted to the further steps of (iii) adsorbing the eluate on a polymeric or ion exchange resin or on carboxymethylcellulose, the adsorbant being different from that used in step (i), and (iv) de-adsorbing the further purified anthracycline glycoside.

By correct selection of the various types of resin according to the raw product to be purified, and the sequence in which they are to be used, a high degree of purity (97 to 98%) in the final product can be obtained, with excellent yields and without the use of chlorinated organic solvents.

The following Examples illustrate the invention.

EXAMPLE 1

Purification of 4-demethoxy-daunorubicin

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15.0 g of raw 4-demethoxy-daunorubicin, having a titre of 70.2% and an impurity content of 18%, were dissolved in 3600 ml of a 0.5% sodium acetate solution. The solution, brought to pH 4.7 by addition of acetic acid, was adsorbed on 400 ml of Rohm and Haas Amberlite XAD2 resin in a column having a diameter of 2.5 cm. "Amberlite" is a Trade Mark. The product was washed with 1000 ml of water and then eluted with a water: methanol mixture (5:1 by volume). As a first eluate, 2000 ml of solution containing aglycones and various other impurities was collected.

Elution was continued with a water:methanol mixture (1:1 by volume) and a 4500 ml fraction was collected. This fraction contained the 4-demethoxy-daunorubicin in solution along with 10% of impurities.

The eluate was brought to pH 2.8 by addition of hydrochloric acid and then vacuum concentrated to a volume of 1500 ml. By addition of sodium acetate to the concentrated solution, the pH was brought to 4.0 and the slightly acidic solution was adsorbed on 150 ml of Pharmacia CM Sepharose Cl 6B resin, contained within a column having a diameter of 2.5 cm and a flow rate of 150 ml/h. "Sepharose" is a Trade Mark. Upon completion of adsorption, the column was first washed with 450 ml of water and then eluted with 0.03%

hydrochloric acid.

The first 800 ml of eluate, containing 18% of impurities was brought to pH 4.7 by addition of sodium acetate and collected for recycling to the first purification stage.

- 5 The successive eluate (3500 ml), containing purified 4-demethoxydaunorubicin, was vacuum concentrated to a volume of 50 ml. 250 ml of acetone was added, and the resultant precipitate was filtered off, washed with acetone and dried. 4-Demethoxy-daunorubicin having a
- 10 titre of 97% and an impurity content of less than 3% was obtained in 52% yield, calculated on the raw starting material.

EXAMPLE 2

Purification of daunorubicin

- 15 15.0 g of daunorubicin hydrochloride having a titre of 74.2% and an impurity content of 8.5%, were dissolved in 4500 ml of water. The solution was brought to pH 5.0 by addition of sodium acetate and adsorbed on 400 ml of Kastell S112 resin or on Rohm and Haas
- 20 Amberlite ER180 resin in a column having a diameter of 2.5 cm and a flow rate of 600 ml/h. 'Kastell' is a Trade Mark.

The product was washed with 1000 ml of a 1% sodium chloride solution and then eluted with a water:ethanol

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mixture (1:1 by volume). The first fraction (400 ml) containing the aglycones in solution was removed and elution was continued to collect 260 ml of eluate containing purified daunorubicin. The pH was adjusted to 2.5 by addition of dilute hydrochloric acid, acetone was added and the product was allowed to crystallize at a temperature of +5°C for 6 hours. The product was filtered, washed with acetone and vacuum dried for 12 hours.

Daunorubicin was obtained in 80% yield, calculated on the raw starting material. As determined by HPLC, its titre was 97% and its impurity content was 2.6%.

EXAMPLE 3

Purification of 4-demethoxy-doxorubicin

2000 ml of aqueous solution containing 10.4 g of 4-demethoxy-doxorubicin with an impurity content of 14% were adsorbed on 50 ml of Rohm and Haas ER180 resin contained within a column having a diameter of 2.5 cm and a flow rate of 250 ml/h.

The eluate, as partially purified by selective adsorption of the impurities, was then adsorbed on 200 ml of Pharmacia CM Sepharose Cl 6B resin. The product was eluted with a methanol:water:concentrated hydrochloric acid mixture (50:50:0.015 by volume),

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discarding the head fractions (about 800 ml) containing aglycones and other impurities. 2000 ml of eluate was then collected, containing the purified substances, and vacuum concentrated to 60 ml. Under stirring over 3 hours, 300 ml acetone were added. The resultant precipitate was filtered off, washed with acetone and dried. 6.5 g of 4-demethoxy-doxorubicin were obtained (yield 58.2% of theoretical) with an impurity content of 3%.

10 EXAMPLE 4

Purification of 4'-deoxy-doxorubicin

15 15.0 g of 4'-deoxy-doxorubicin, having a titre of 69.2%, an organic impurity content of 12% and a mineral salt impurity content of 12%, were dissolved in 2000 ml of water and adsorbed on 300 ml CM Sephadex C25 resin at a flow rate of 600 ml/h. 'Sephadex' is a Trade Mark. Upon completion of adsorption, the solution was eluted with 0.03% hydrochloric acid and a fraction (6500 ml) was obtained containing 4'-deoxy-doxorubicin.

20 This fraction, brought to pH 3.8 by addition of a solution of sodium hydrate, was adsorbed on 200 ml of Kastell S112 resin at a flow rate of 400 ml/h. The product was washed with 600 ml of water and then eluted with methanol acidified to pH 2.0 by hydrochloric acid. 600 ml of core eluate were obtained, and vacuum

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concentrated to 60 ml. Under stirring, the concentrated solution was slowly poured into 600 ml acetone. The precipitate formed was filtered off, washed with acetone and dried. 4'-deoxy-doxorubicin was obtained (yield 5 61% of theoretical) with a titre of 95.8% and an impurity content of 4.2%.

EXAMPLE 5

Purification of 4'-epi-doxorubicin

15.0 g of raw 4'-epi-doxorubicin, having a titre of 10 75.9% and an impurity content of 15%, were dissolved in 4000 ml of water. The solution was brought to pH 4.8 by addition of sodium formate and adsorbed on 400 ml of Amberlite IRC 724 resin contained within a column having a diameter of 2.5 cm and a flow rate of 1600 15 ml/h. The column was washed with 800 ml water and then eluted with a methanol:water:concentrated hydrochloric acid mixture (95:5:0.015 by volume). 4000 ml of core eluate were collected, containing 4'-epi-doxorubicin still with 10% impurities, and 1500 20 ml of tail eluate mainly containing impurities were also collected.

The core eluate was concentrated to 1500 ml, brought to pH 4.8 by addition of sodium formate and adsorbed on 250 ml Whatman carboxymethylcellulose resin 25 contained within a column having a diameter of 2.5 cm

and a flow rate of 500 ml/h. 'Whatman' is a Trade Mark.
Upon completion of adsorption, the product was washed with
an ethanol: water: concentrated hydrochloric acid mixture
(99.3:0.7:0.015 by volume) and then eluted with an
5 ethanol:water:concentrated hydrochloric acid mixture
(90:10:0.05 by volume) to collect 3200 ml of core eluate.
The eluate was vacuum concentrated to 60 ml and the
product was precipitated by addition of 300 ml of
acetone. 6.9 g of 4'-epi-doxorubicin were obtained,
10 having a titre of 91.2% and an impurity content of
3%. Yield = 55.3%.

EXAMPLE 6

Purification of doxorubicin

(a) 70 g of doxorubicin were dissolved in 28 litres of
15 water. The pH was brought to 3.7-4.5 by addition of
buffer and the solution was adsorbed on 2 litres of
Kastell S112 resin contained within a column having a
diameter of 6 cm. The product was eluted with a
mixture of 35 litres of water and 15 litres of methanol.
20 The eluate (40 litres) was vacuum concentrated to
500 ml and the product was crystallized by addition of a
mixture of 1 litre of ethanol and 4.5 litres of acetone
under stirring at +5°C for 3 hours. The product was
filtered off, washed with 0.01 acetone and vacuum dried
25 at +40°C for 5 hours.
56.0 g of purified doxorubicin were obtained from this
first step.

(b) 80.0 g of doxorubicin, purified as described in part (a) of this Example, were dissolved in 24 litres of water and brought to pH 4.0 by addition of buffer. The solution was adsorbed on a column having a diameter of 12 cm and containing 1.6 litres of Whatman carboxymethylcellulose resin. The column effluent was removed. After washing with 3.2 litres of water, the product was eluted with 55 litres of water brought to pH 2.5 by addition of hydrochloric acid. 46 litres of eluate were collected and vacuum concentrated to a volume of 600 ml. The product was allowed to crystallize by cautious addition of an isopropanol:acetone mixture (1:3 by volume). The product was filtered off, washed with 1 litre of acetone and dried at +40°C for 4 hours. 65 g of doxorubicin were obtained with a titre of 98.5% and an impurity content of 1.5%.



CLAIMS

1. A process for the purification of a raw anthracycline glycoside, obtained by fermentation or synthetically, the process comprising (i) adsorbing an aqueous solution having a pH of from 3 to 5 of the raw
5 anthracycline glycoside on a polymeric or ion exchange adsorbing resin or on carboxymethylcellulose and (ii) de-adsorbing the purified anthracycline glycoside by elution with water or with slightly acidic water or with a mixture of water and a polar solvent or with a
10 mixture of slightly acidic water and a polar solvent .
 2. A process according to claim 1 further comprising (iii) adsorbing the eluate on a polymeric or ion exchange resin or on carboxymethylcellulose, the
15 adsorbant being different from that used in step (i), and (iv) de-adsorbing the further purified anthracycline glycoside.
 3. A process according to claim 2 in which the eluate
20 from step (ii) is brought to a pH of from 3 to 5 before carrying out step (iii).
 4. A process according to claim 2 or claim 3 in which the elution of step (iv) is carried out using
25 slightly acidic water or a mixture of water and a polar solvent or a mixture of slightly acidic water and a polar solvent.
 5. A process for the purification of a raw
30 anthracycline glycoside, obtained by fermentation or synthetically, the process being substantially as described herein with reference to any of the Examples.
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