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Purification of an ascomycin derivative with simulated moving bed chromatography. A case study

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Abstract

A purification process was developed for the separation of a semi-synthetic ascomycin derivative from its by-products. The process consists of a silica gel filtration and crystallization step prior to two simulated moving bed (SMB) separations, where in the first part the polar by-products and in the second part the apolar by-products were removed. The desired purity was achieved in a final crystallization step. Key elements of the whole process were the design of the first crystallization to obtain a product feasible for SMB chromatography and the specification of operating parameters for the two corresponding SMB steps. Starting from a crude product with an assay of only 44.9% an overall yield for the whole process of 81.0% was achieved with a final purity of >98%.

Keywords: Simulated moving bed; Ascomycin

1. Introduction

The ascomycin derivative 9-ethyl-6,16,20-trihydroxy-4-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-15,17-dimethoxy-5,11,13,19-tetramethyl-3-oxa-22-azatricyclo[18.6.1.0^{1,22}]heptacos-10-ene-2,8,21,27-tetraone (the structure is given in Fig. 1 and the compound is further abbreviated AD) is a novel anti-inflammatory drug substance. It is proposed for treatment of atopic dermatitis, psoriasis and allergic dermatitis. The drug substance is manufactured by fermentation of ascomycin, chemical mediated cyclisation (KOH in acetonitrile) and final purification with chromatography and crystallization [1]. The purity profile after the cyclisation step is given in Fig. 2 and the structures of the most important by-products 19-Epi-AD, 9-Epi-AD and ascomycin in Fig. 1. The goal of the process

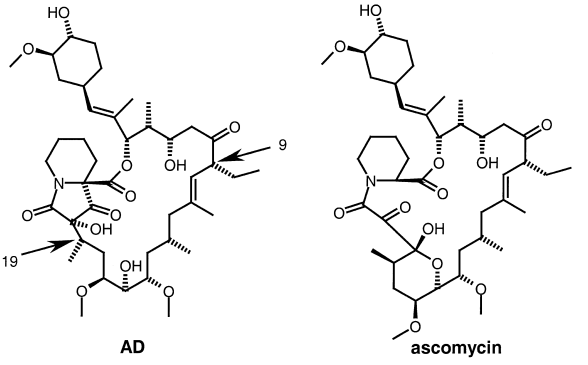

Fig. 1. Structures of AD and ascomycin (in the case of 9-Epi-Ad and 19-Epi-AD, the absolute configuration of C-9 and C-19, respectively is opposite).

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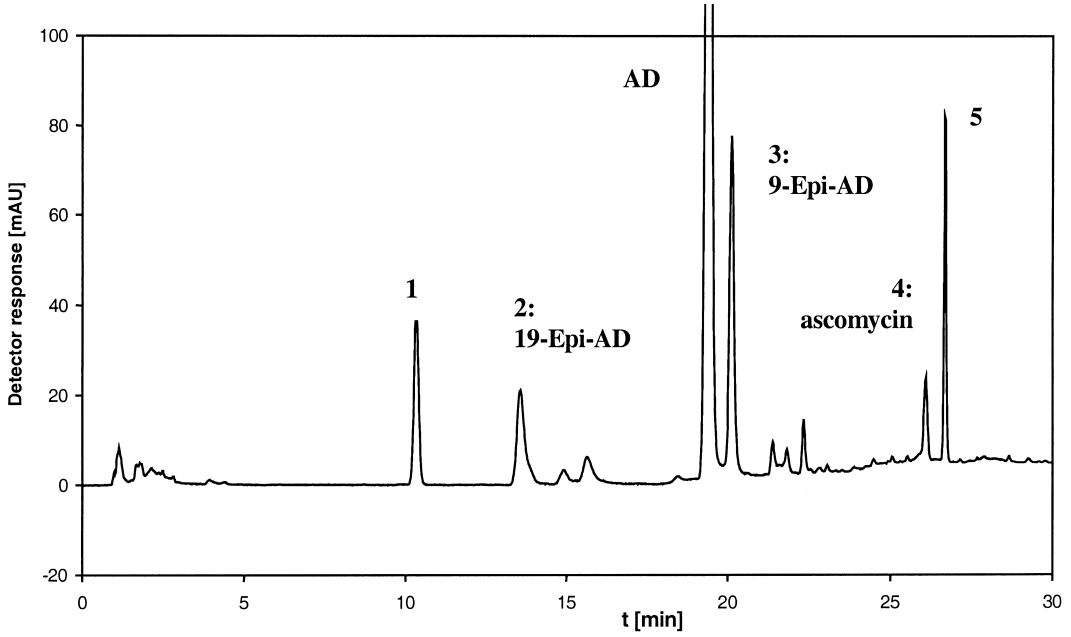

Fig. 2. HPLC analysis of crude AD after chemical mediated cyclization of ascomycin. The assay for AD is only 44.9% and the values for the main critical impurities are as follows: 19-Epi-AD (5.4 area%), 9-Epi-AD (10.9 area%) and ascomycin (2.8 area%). For chromatographic conditions see Experimental section.

described in the following is the removal of the above mentioned by-products to obtain a final purity of >98%. Since it has been demonstrated in previous manufacturing campaigns, that silica gel chromatography is facultative for the removal of 9-Epi-AD, the basic intention was to consequently substitute this purification step with simulated moving bed (SMB) chromatography to obtain higher production rates.

2. Experimental

2.1. Materials

AD is an active ingredient from Novartis Pharma (Basel, Switzerland). Zorbax LP (40 μm, 100 Å) was supplied from DuPont (Bad Homburg, Germany) and silica gel (Merck, 70–230 mesh ASTM) from Merck (Darmstadt, Germany). Ethyl acetate, hexane, acetone, methanol, *tert*.-butyl methyl ether and ethyl methyl ketone for preparative purposes are of technical quality and were supplied from the pilot plants from Novartis Pharma.

2.2. Silica gel filtration (step 1)

A 700-g amount of silica gel (Merck, 70–230 mesh ASTM) is filled in a glass column (30 cm×12 cm I.D.) and wet with 1 l of ethyl acetate–methanol (95:5, v/v) which leads to a corresponding bed height of 14 cm. A 233-g amount of crude AD (assay: 44.9%) is dissolved in 2 l of ethyl acetate–methanol (95:5, v/v), poured onto the column and afterwards washed out (with 6.5 l of solvent mixture as above) for 2.5 h with a flow-rate of 40 ml/min. The eluate is evaporated to dryness yielding 174.8 g of prepurified AD (product of step 1) with an assay of 58.9%. The purity profile is given in Fig. 3.

2.3. Crystallization (step 2)

Prepurified AD (product from step 1) is dissolved in the 25-fold amount (%, w/w) of *tert*.-butyl methyl

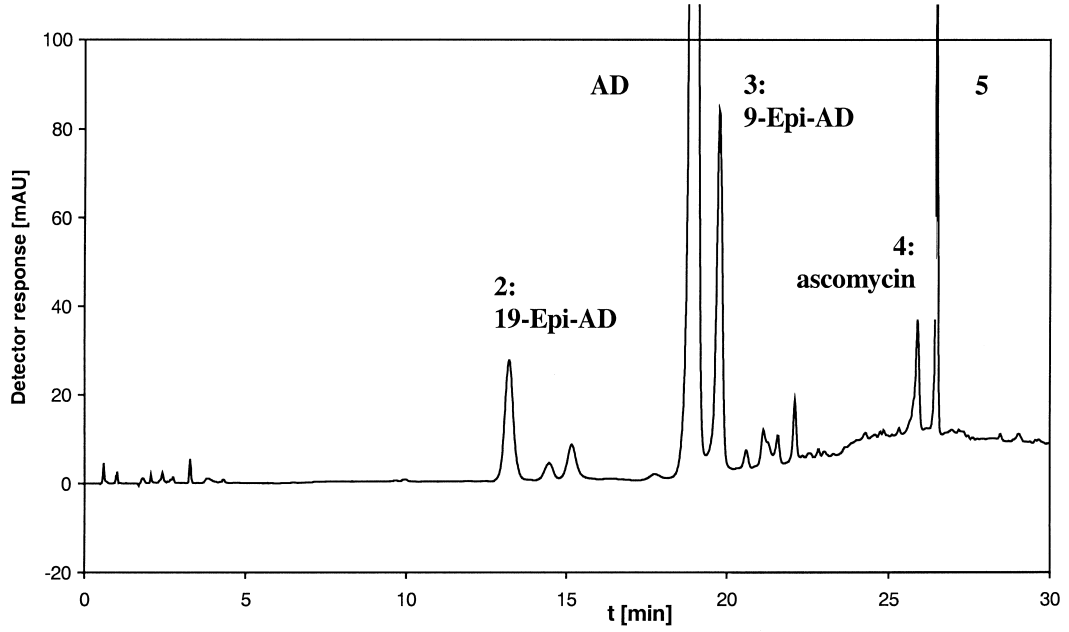

Fig. 3. HPLC analysis of prepurified AD after silica gel filtration. The assay for AD is 58.9% and the values for the main critical impurities are as follows: 19-Epi-AD (5.8 area%), 9-Epi-AD (12.0 area%) and ascomycin (2.9 area%). For chromatographic conditions see Experimental section.

ether–ethyl acetate (1:1, v/v) at 25°C. The solution is cooled down to 0°C during 1 h accompanied by the rapid crystallization of 19-Epi-AD. The solution is further stirred for 20 h at 0°C and afterwards filtered. The solution is evaporated to dryness yielding 98.4% of AD with a content of 1.1 area% of 19-Epi-AD. The purity profile of crystallized AD (product from step 2) is given in Fig. 4.

2.4. Simulated moving bed chromatography (steps 3 and 4)

The equipment used in our study was identical with the one described by Negawa and Shoji [2], with the exception that 12 columns (with three columns per section) instead of eight have been used. The 12 separation columns were slurry packed in methylene chloride with silica gel (40 μm) and connected to rotary valves (12-port VICI). Each column was 10 cm×2.0 cm I.D. The electric actuated rotary valves were controlled by a personal computer using laboratory-developed software. The feed and raffinate pump was a Jasco PU-980, the desorbent pump a Jasco PU-986 and the recycle pump was a Jasco PU-987.

Conditions for step 3 were as follows: stationary phase: Zorbax LP (40 μm, 100 Å). Mobile phase: ethyl acetate–hexane–acetone (75:14:11, v/v/v). Feed concentration: 25 g/l. Feed flow: 0.5 ml/min. Eluent flow: 15 ml/min. Extract flow: 11.5 ml/min. Raffinate flow: 4.0 ml/min. Switching time: 7 min.

Conditions for step 4 were as follows: stationary phase: Zorbax LP (40 μm, 100 Å). Mobile phase: ethyl acetate–hexane–acetone (75:14:11, v/v/v). Feed concentration: 50 g/l. Feed flow: 0.5 ml/min. Eluent flow: 17.5 ml/min. Extract flow: 8.0 ml/min. Raffinate flow: 10.0 ml/min. Switching time: 7 min.

2.5. Final crystallization (step 5)

A 3.0-g amount of pre-purified AD (product of step 4) is dissolved in ethyl methyl ketone at 63°C and stirred for 10 min. Afterwards 3.6 ml heptane is slowly added within 15 min to the solution which is then kept for a further 1.5 h at room temperature for crystallization. The suspension is then kept again for

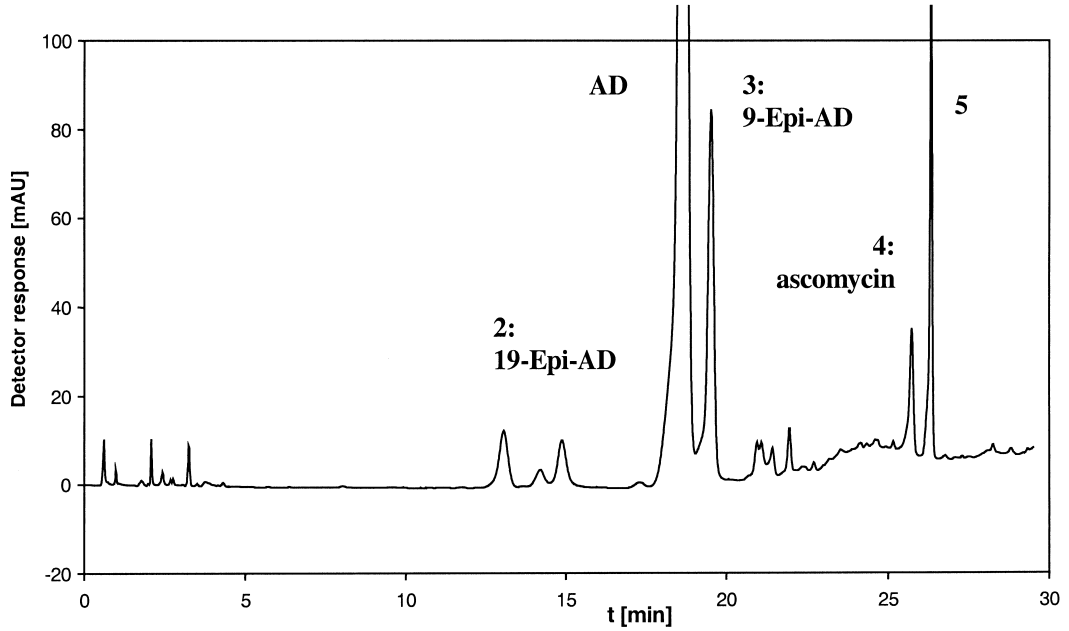

Fig. 4. HPLC analysis of a non-optimized crystallization of 19-Epi-AD. The assay for AD is 59.2% and the values for the main critical impurities are as follows: 19-Epi-AD (2.5 area%), 9-Epi-AD (11.9 area%) and ascomycin (2.6 area%). For chromatographic conditions see Experimental section.

1.5 h at 1°C. Finally, the crystals are filtered, washed and dried under vacuum.

2.6. High-performance liquid chromatography (HPLC) analysis

A Hewlett-Packard 1100 system with UV detection was used. The analytical separations were performed on a YMC ODS AM silica gel (5 μm) analytical column (250 mm×3 mm I.D.). A water–acetonitrile gradient was used in the following manner for the elution of AD and its by-products: solvent A: water–acetonitrile (9:1, v/v). Solvent B: water–acetonitrile (2:8, v/v).

Time (min)	Solvent A (%)	Solvent B (%)
0	50	50
15.0	50	50
25.0	0	100
30.0	0	100

The flow-rate was kept at 1.0 ml/min and the column temperature at 70°C. UV detection was performed at 205 nm and the injected volume was 10 μl (concentration: 10 mg/10 ml acetonitrile).

2.7. Determination of adsorption isotherms

An artificial test mixture containing 86.4% AD, 8.4% 9-Epi-AD and 5.2% remaining by-products was chosen for the application of the adsorption–desorption method. Since solute concentrations for the components of the mixture were simply calculated from relative peak areas of analytical chromatograms the pre-purified mixture was used for the measurements in order to improve the accuracy of quantification. A series of 12 adsorption–desorption experiments was performed. Solutions of known concentrations in the range of 0.1 g/l up to 10 g/l in ethyl acetate–hexane–acetone (75:14:11, v/v/v) were pumped through the column until equilibrium was reached. The corresponding amounts of solutes in the solid-phase were obtained after desorption

with the same solvent mixture and quantitative HPLC analysis of the collected desorbate. Reaching of equilibrium stages was monitored with a differential refractometer (Knauer WellChrom K-2300) indicated by a plateau in the chromatogram. The measurements were performed on a cited SMB column (10 cm×2.0 cm I.D, packed with silica gel, 40 μm) at 23°C.

3. Results and discussion

3.1. Strategy

The quality of crude AD after chemical cyclization is given in Fig. 2. The following purification strategy (see Table 1) was chosen to at least obtain drug substance with a purity of >98%. A silica gel filtration prior to normal-phase chromatography is generally preferred, because an effective removal of strong polar by-products during the filtration procedure guarantees faster run times for batch elution chromatography (and shorter cycle times in the SMB mode, respectively) and a minimum time for column regeneration. Our first SMB experiments failed, because of crystallization of 19-Epi-AD in the system. This was a result of higher concentrations in the SMB unit in comparison to batch elution chromatography, where this problem never appeared. A tailor-made crystallization procedure could be elaborated, that selectively removed 19-Epi-AD with a high yield. In the following SMB chromatography (step 3, Table 1) the operating conditions were designed to selectively remove 9-Epi-AD, being the most critical separation problem in batch elution chromatography. It is important to know that 9-Epi-AD always elutes after AD in normal-phase mode as well as in reversed-phase chromatography. In the second SMB purification (step 4, Table 1) ascomycin and other unpolar by-products are removed and finally, the AD enriched extract is crystallized (step 5, Table 1).

Table 2
Yields for the purification of crude AD after chemical cyclization

Step	Technique	Yield (%)
1	Silica gel filtration	99.0
2	Crystallization	98.4
3	SMB-chromatography A	92.0
4	SMB-chromatography B	99.5
5	Final crystallization	93.0
	Overall	81.0

3.2. Silica gel filtration (step 1)

The silica gel filtration should not be considered as a chromatography, since the product is only being pumped through a silica gel column (see Experimental) without any fractionation. The purity profile of silica gel filtrated material is shown in Fig. 3 demonstrating that by-product 1 and more polar by-products (Fig. 2) are being quantitatively removed. Nevertheless, the recovery for AD (see Table 2) is extremely high (yield 99.0%).

3.3. Crystallization (step 2)

As has been pointed out in the Strategy section, the removal of 19-Epi-AD is a prerequisite for the SMB runs, because this epimer tends to crystallize in the chromatographic system. Careful evaluation of the crystallization conditions finally revealed a crystallization procedure, that decreases the content of 19-Epi-AD from 8.4 to 1.1 area% without any loss of AD (yield: 98.4%). The solvent system *tert*.-butyl

Table 1
Purification strategy for crude AD after chemical cyclization

Step	Technique	Aim of the purification
1	Silica gel filtration	Removal of very polar by-products
2	Crystallization	Removal of 19-Epi-AD
3	SMB-chromatography A	Removal of 9-Epi-AD
4	SMB-chromatography B	Removal of ascomycin and unpolar by-products
5	Final crystallization	Final purity of AD>98%

methyl ether–ethyl acetate (1:1, v/v) fulfilled the requirements (good solubility for AD and bad solubility for 19-Epi-AD) best. In addition, it turned out that concentration and temperature also played an important role to design a tailor-made process which led to a high recovery yield as well as to a low content of 19-Epi-AD. A second crystallization was not necessary, since it could be demonstrated that the SMB process tolerates 19-Epi-AD up to 2.0%. The purity profile of a preliminary crystallization experiment is given in Fig. 4 where the content of 19-Epi-AD was reduced under non-optimized conditions from 5.8 to only 2.5 area%. The comparison of the chemical purities in Figs. 3 and 4 also underlines, that the removal of 9-Epi-AD and ascomycin is not easily achieved with crystallization methods and asks for chromatographic solutions.

3.4. SMB chromatography A (step 3)

SMB chromatography has emerged as a promising technology allowing the continuous counter-current separation of a mixture into two streams of products. The process, developed in the early 1960s [3], has been used for many years in the petrochemical [4,5] and sugar industries [6,7] for the large-scale separation of binary mixtures. Recently, the growing demand for efficient methods to purify optical isomers promotes the application of the SMB principle for more difficult enantioseparations (again binary mixtures), in the pharmaceutical industry [8–16]. Less applications are known where a product is purified from a multi-component mixture, as has been demonstrated for the purification of cyclosporin A from fermentation brew [17]. The principle of the SMB separator has been introduced and discussed in detail in the above mentioned papers and is therefore not subject of the present publication.

The equilibrium–dispersive model [18] can be applied to predict the concentration profiles in all SMB columns [19]. Assuming that: (i) there is a permanent equilibrium between the liquid and the solid phases; and (ii) the contributions to band broadening caused by axial dispersion and mass transfer resistances can be described by an apparent dispersion coefficient, D_{ap}, as a lumped parameter, the following mass balance Eq. (1) of a component i has to be solved for each column j of the SMB unit:

$$\frac{\partial C^j_i}{\partial t} + \left(\frac{1-\epsilon}{\epsilon}\right)\frac{\partial q^j_i}{\partial t} + u_j\frac{\partial C^j_i}{\partial z} = D_{\mathrm{ap}_i}\frac{\partial^2 C^j_i}{\partial z^{j^2}},$$
$$i = 1, \ldots, N_{\mathrm{Comp}},\ j = 1, \ldots, N_{\mathrm{Col}} \quad (1)$$

In this equation ϵ is the total porosity of the columns and u_j is the linear velocity of the liquid phase in column j. The concentrations of component i in the liquid and in the solid phases, C_i and q_i, respectively, are related through the adsorption isotherms.

Several methods are known for the determination of adsorption isotherms [20]. For our problem, the separation of AD from 9-Epi-AD with SMB chromatography, the adsorption–desorption method has been chosen. After saturation of the column with defined solute concentrations C_{E_i} in the range of 0.1 g/l up to 10 g/l in ethyl acetate–hexane–acetone (75:14:11, v/v/v) the corresponding amounts of solutes m_i in the column of volume V are obtained after desorption in each step with the same solvent mixture. Equilibrium conditions assumed the corresponding concentrations in the stationary phase q_{E_i} are obtained according to Eq. (2):

$$q_{\mathrm{E}_i}(C_{\mathrm{E}_1}, C_{\mathrm{E}_2}, \ldots, C_{\mathrm{E}_N}) = \frac{m_i - \epsilon V C_{\mathrm{E}_i}}{(1-\epsilon)V},$$
$$i = 1, \ldots, N \quad (2)$$

To model the adsorption equilibrium a suitable competitive isotherm equation has to be chosen. The following multi-Langmuir, Eq. (3), was found to represent the experimental data satisfactorily:

$$q_i = \frac{a_i C_i}{1 + \sum_{j=1}^{N} b_j C_j},\ i = 1, \ldots N \quad (3)$$

The free parameters in Eq. (3) a and b were obtained by non-linear regression using Marquardt's method [21] minimizing an overall objective function including all data available.

Since the separation of 9-Epi-AD from AD is the most critical separation problem, the determination of the adsorption isotherms has been concentrated on only these two components for the first SMB chromatography step. As described in the Experimental

section a pre-purified test mixture with a different composition than the feed has been chosen for this purpose. However, by using the universal multi-Langmuir isotherm equation for modeling of the adsorption behavior the correct description of the interactions between the two main components AD and 9-Epi-AD is independent of the composition of the test mixture, a negligible influence of the remaining components supposed. The procedure for the adsorption–desorption process has been described in the Experimental section and the results are shown in Fig. 5. With the experimental values given in Fig. 5 the free parameters, *a* and *b*, in the multi-Langmuir Eq. (3) have been fitted as described above. The results of this investigation are summarized in Table 3.

Table 3
Adsorption isotherm parameters determined for AD and 9-Epi-AD according to the multi-Langmuir Eq. (3)

Parameter	AD	9-Epi-AD
a (l/l)	7.266	9.975
b (l/g)	0.05601	0.00089

Due to the non-linear character of the isotherm equation, the solution of Eq. (1) requires the use of numerical methods. The fast and stable finite difference algorithm as implemented in the software SMB-Guide (Knauer, Berlin, Germany) was used for our calculations. Further details concerning the numerical solution are reported elsewhere [22]. The SMB-Guide software is able to predict the region of complete separation [23–26] on the basis of known Langmuir or multi-Langmuir isotherms or even for the bi-Langmuir and multi-bi-Langmuir model. Fig. 6 illustrates the internal concentration profiles for AD and 9-Epi-AD along the columns of the SMB obtained for the operating conditions mentioned in the Experimental section after reaching steady-state conditions in the system. The predicted profiles are

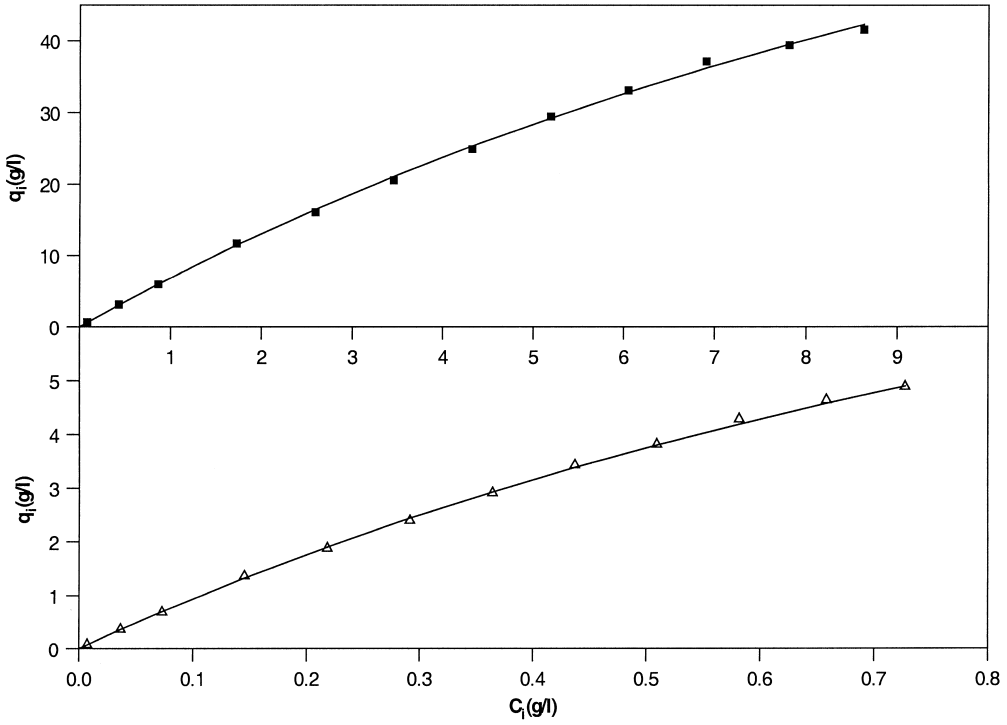

Fig. 5. Competitive adsorption isotherms of AD (■) and 9-Epi-AD (△) determined for the artificial test mixture containing 86.4 area% AD, 8.4 area% 9-Epi-AD and 5.2 area% remaining by-products. Experimental (dots) and fitted (curves) values. The parameters of the applied isotherm equation are given in Table 3.

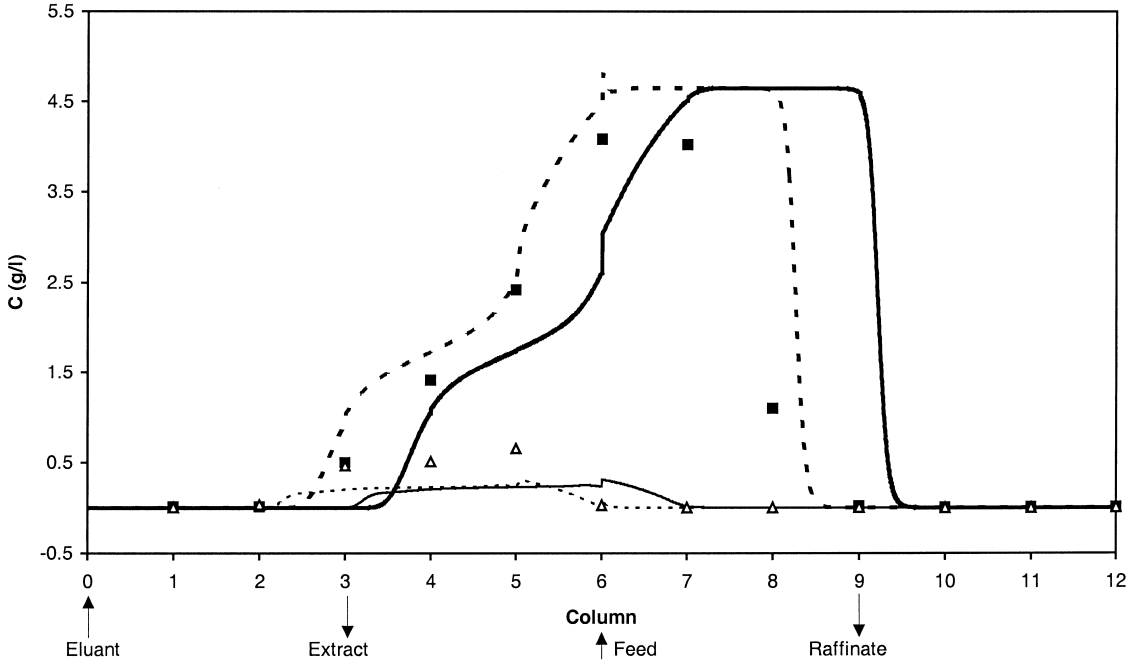

Fig. 6. Experimental and predicted internal concentration profiles for the separation of AD and 9-Epi-AD using the operating conditions mentioned in the Experimental section (step 3). Experimental data [AD: (■), 9-Epi-AD: (△)] were taken at the half-time period in the cyclic steady state. Theoretical data (AD: thick lines, 9-Epi-AD: thin lines) illustrate the band profiles along the columns just after switching (dotted) and just before switching (solid). Calculations are based on the parameters given in Table 3.

shown just after and before a switching operation, performed periodically with the time t_{shift}. The experimental data were measured at $t_{shift}/2$. Although the values show some scatter, the general shape of the experimental profiles is well represented by the model predictions. This result is confirmed by comparing experimental with predicted performance parameters as given in Table 4, with the exception that the yield for AD is a little bit higher than expected. Fig. 7 outlines a typical purity pattern of recovered AD from the raffinate prior to step 4.

Table 4
Experimental vs. predicted results for the SMB separation of AD and 9-Epi-AD (step 3)

	Experiment	Modeling
Concentration extract: 9-Epi-AD (g/l)	0.122	0.111
Concentration raffinate: AD (g/l)	1.59	1.44
Yield AD (%)	93.1	80.6
Purity raffinate (%)	99.7	100
Production rate (g AD/h/kg silica gel)	2.07	1.80
Solvent consumption (l/g AD)	2.33	2.69

3.5. SMB chromatography B (step 4)

In the second SMB purification step, only the flow-rates are adjusted to simply remove ascomycin from AD now being concentrated in the extract stream. Since the separation of AD from ascomycin is less difficult one can omit the determination of the adsorption isotherm of ascomycin. In such a case the operating conditions can easily be chosen with the help of a simple chart that depicts visually the SMB design criteria [27]. The quantitative removal of ascomycin from purified AD (product from step 3) is shown in Fig. 8, illustrating the purity profile of the extract stream in steady state. Even under non-optimized conditions an excellent yield of 99.5% can easily be achieved. Final optimization work is there-

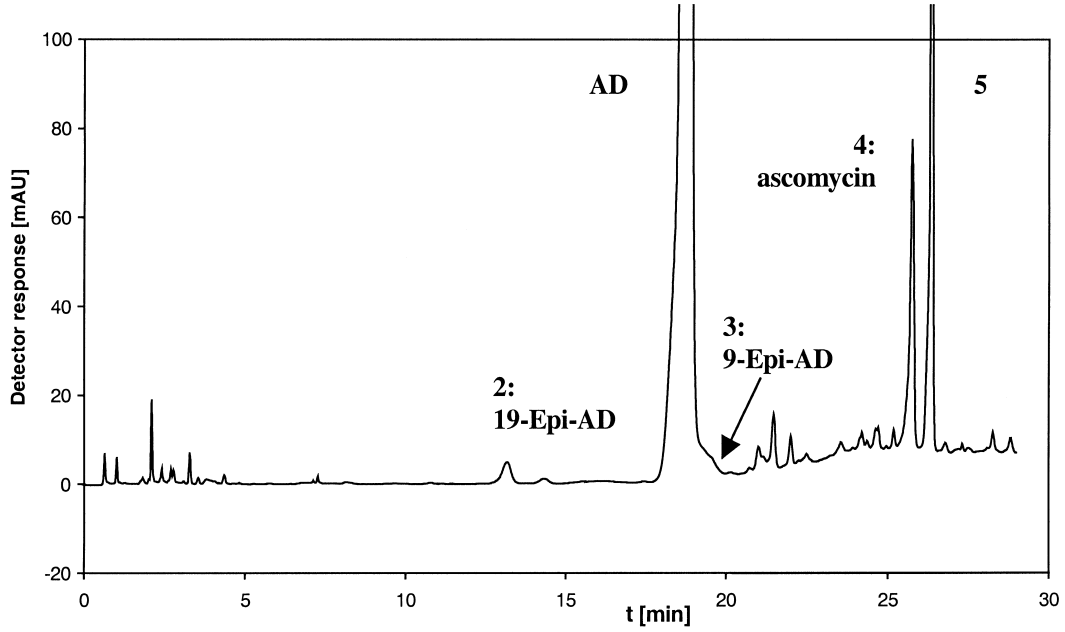

Fig. 7. Typical HPLC chromatogram of the raffinate (cycle 30) from the first SMB chromatography (step 3), removal of 9-Epi-AD in steady state. The assay for AD is 75.1% and the values for the main critical impurities are as follows: 19-Epi-AD (0.5 area%), 9-Epi-AD (0.6 area%) and ascomycin (4.6 area%). For chromatographic conditions see Experimental section.

fore restricted to increase the production rate and to further reduce the solvent consumption.

3.6. Final crystallization (step 5)

Although the extract product from step 4 fulfils the requirement of >98% purity, it is finally crystallized to obtain a product with a desired particle size. During this final purification step, the amount of 9-Epi-AD is again reduced to 0.05% and the amount of ascomycin to 0.04%, respectively. The by-products X and Y in Fig. 8, being ascomycin derivatives, are not removed during the crystallization and previous performed purification procedures. Their amount in AD corresponds with the quality of the ascomycin raw material from the fermentation process. It is not necessary to remove them, because their occurrence and amounts can be limited during the work up of the fermentation process. Nevertheless, as a back up variant, it is possible to remove them during the second SMB chromatography (step 4).

4. Conclusion

A purification process was developed for the separation of the semi-synthetic ascomycin derivative AD from its by-products. The key-element of the whole process is the removal of 9-Epi-AD which was eliminated by preparative liquid chromatography in previous manufacturing campaigns. On the basis of determined adsorption isotherms for AD and 9-Epi-AD this batch elution process step could be replaced by an SMB chromatography allowing the continuous purification of AD with increased production rates and lower solvent consumption. The adsorption–desorption procedure was applied successfully for the determination of the competitive adsorption isotherms. Modeling of the the first SMB process could be achieved by applying the software package SMB-Guide (Knauer), which was found to be suitable to predict the mentioned flow-rates in the system. As a prerequisite for the first SMB chromatography, 19-Epi-AD (which tends to crystallize in the equipment at higher concentrations) had to be

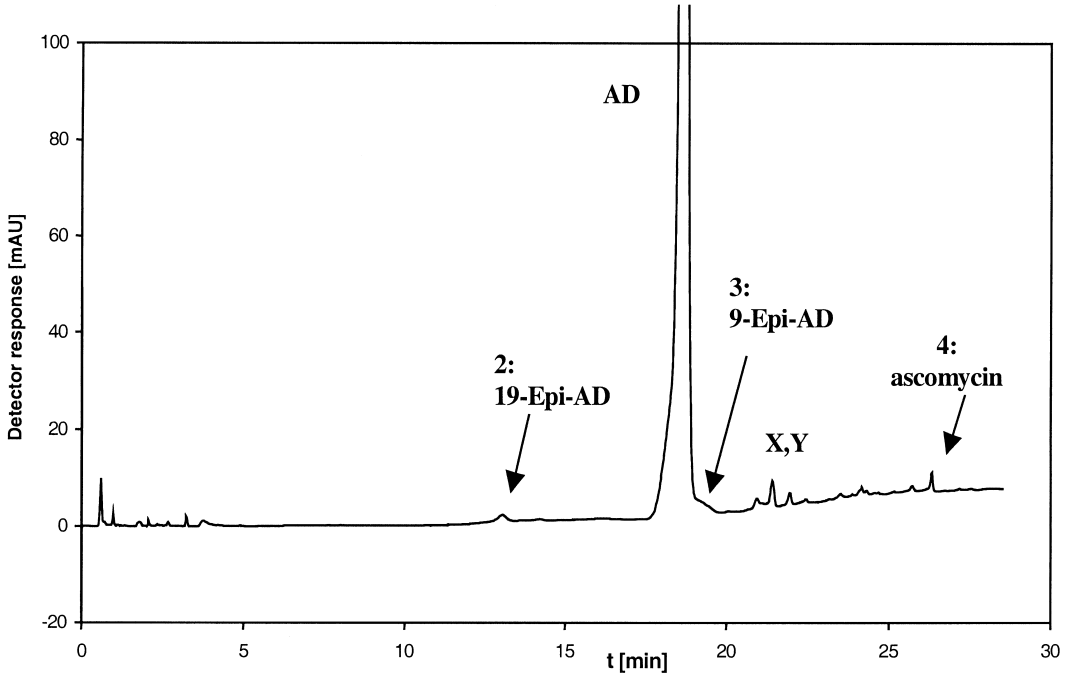

Fig. 8. Typical HPLC chromatogram of the extract (cycle 13) from the second SMB chromatography (step 4), removal of ascomycin in steady state. The purity profile for the main critical impurities is as follows: 19-Epi-AD (0.3%), 9-Epi-AD (0.6%) and ascomycin (0.1%). For chromatographic conditions see Experimental section.

removed. This could easily be achieved in a previous performed tailor-made crystallization. Starting from a crude product with an assay of only 44.9% an overall yield for the whole process of 81.0% could be achieved with a final purity of >98%.

5. Symbols

a	Means Parameter in isotherm equation, Eq. (3)
b	Means Parameter in isotherm equation, Eq. (3)
C	Means Liquid phase concentration
C_E	Means Liquid phase concentration at equilibrium
D_{ap}	Means Apparent dispersion coefficient
m	Means Mass
N_{Col}	Means Number of columns
N_{Comp}	Means Number of components
q	Means Solid phase concentration
q_E	Means Solid phase concentration at equilibrium
t	Means time
t_{shift}	Means Switching time
u	Means Linear velocity
V	Means Volume of the column
z	Means Axial coordinate of the column
Subscripts	
i	Means Component
j	Means Column
Greek	
ϵ	Means Total porosity

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