HUNGARIAN JOURNAL
OF INDUSTRIAL CHEMISTRY
VESZPRÉM
Vol. 34. pp. 15-20 (2006)

QUASI-CONTINUOUS ELUTION CHROMATOGRAPHIC PURIFICATION OF A STEROID ACTIVE COMPOUND

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A pilot-scale preparative batch elution chromatographic separation of a steroid active compound from its impurities was developed and is applied at our company. The process consists of the following steps: (1.) injection of the crude sample solved in the eluent onto the chromatographic column, (2.) elution of the less retained impurities with the eluent, (3.) elution of the pure fraction of the active compound (main fraction) with the eluent, (4.) back-flushing the column with the more polar solvent component of the eluent to remove the strongly retained compounds, (5.) conditioning the column with the eluent to prepare for the next injection. The above mentioned five-step chromatographic process can be realized quasi-continuously by applying the Simulated Moving Bed (SMB) instrument. The subsequent steps can be carried out in parallel in the zones of the SMB unit, which are independent from each other in this special case.

In our present paper the elaborated quasi-continuous elution chromatographic separation is shown using the following instrument set up and experimental conditions: a KNAUER CSEP 9116 laboratory-scale SMB unit with five KNAUER K-501 HPLC pumps and eight columns (column dimension: 250X16 mm I.D.). The columns were packed in our laboratory by dry-packing method using vibration, the applied packing material was UETIKON C-490 15-35 μm Si gel. The appropriate eluent was methylene chloride/ethyl acetate mixture and as back-flushing solvent, pure ethyl acetate was used. After the elaboration of quasi-continuous elution chromatographic purification, the intensification of the process was carried out. The specific capacity parameters of the best quasi-continuous experiments were compared with the performance parameters of the current batch pilot-scale separation of the compound.

Keywords: quasi-continuous, pilot-scale, preparative chromatographic separation, steroid, Simulated Moving Bed (SMB)

Introduction

In pharmaceutical industry there is a strict demand on the quality of active compounds. In most cases the amount of all impurities must be under 0.5-1 %, the maximum level of individual impurities had to be under 0.1 %. In many cases these demands can not be fulfil with traditional techniques, such as distillation or crystallization. Pilot- and process-scale preparative chromatographic processes can solve these difficult separation problems. [1]

The preparative chromatographic processes can be divided into batch and continuous methods, depending on the actual separation task. In some cases these techniques can compete with each other. [2-5]

The application areas of continuous counter-current separation methods are chiral, isomer, or other two-component (or pseudo two-component) separation problems. [6-11]

The Simulated Moving Bed (SMB) technology is the up-to-date solution for the practical realization of continuous, counter-current preparative chromatographic processes. It was invented and patented in the late 1950's and was mainly applied in the petrochemical and sugar industries for large-scale separations. SMB has been introduced in the pharmaceutical industry since the 1990's. [12]

The principle of the technique has been described in numerous publications [13-15]. The essence of the process is that instead of the continuous transportation of the particulate solid phase, which leads to a lot of difficulties, (bad efficiency because of high HETP and mechanic problems with transportation of the solid phase) the moving of the solid phase in packed columns is simulated. This simulated moving can be solved either by switching the inlets and outlets co-current with the liquid flow direction, or by the real rotation of the packed columns, connected to special distributing valves. *(Fig. 1.)*

In the SMB process the strong (*S*, more retained) and weak (*W*, less retained) components of the feed (*F*) of concentration $c_{F,S}$ and $c_{F,W}$ are separated into two outlet flows. In the extract (*E*, $c_{E,S}$, $c_{E,W}$) the better adsorbing strong, while in the raffinate (*R*, $c_{R,S}$, $c_{R,W}$) the less adsorbing weak components are enriched, respectively. *(Fig. 2.)*

In purification tasks there are usually one (or some more) main compounds in the presence of numerous impurities of small amount. In such cases SMB is generally not applicable, mainly preparative batch elution techniques are used. [16-19]

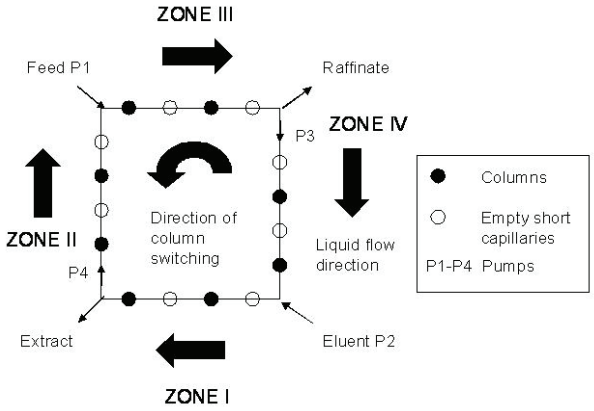

Fig. 1: Conventional four-zone SMB unit with eight columns

The conventional SMB instrument set-up can be utilized to solve a batch elution chromatographic purification task quasi-continuously, if an astute assembling mode of the available unit is applied.

In this present study the quasi-continuous implementation of a current pilot-scale batch elution purification of a steroid active compound is shown. The process consists of the following steps: (1.) injection of the crude sample solved in the eluent onto the chromatographic column, (2.) elution of the less retained impurities with the eluent, (3.) elution of the pure fraction of the active compound (main fraction) with the eluent, (4.) back-flushing the column with the more polar solvent component of the eluent to remove the strongly retained compounds, (5.) conditioning the column with the eluent to prepare for the next injection.

The subsequent steps can be carried out in parallel in the zones of the SMB unit, which are independent from each other in this special case.

The Implementation of Quasi-Continuous Process Starting from Usual SMB Instrument Set-Up

The conventional SMB unit has four zones as can be seen in Fig.1. and Fig.2.

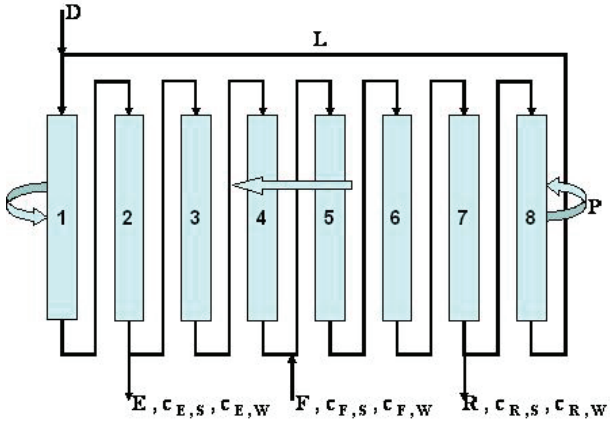

Fig. 2.: One of the applied practical solutions of the Simulated Moving Bed

It is possible to carry out the above-mentioned actual five-step batch elution chromatographic purification procedure quasi-continuously in these zones with the help of the rotating switching valve of the SMB instrument. As in our special case the zones are independent from each other, they can be decomposed to six parts and the flow rate can be reversed in zone I, as *Fig. 3* shows.

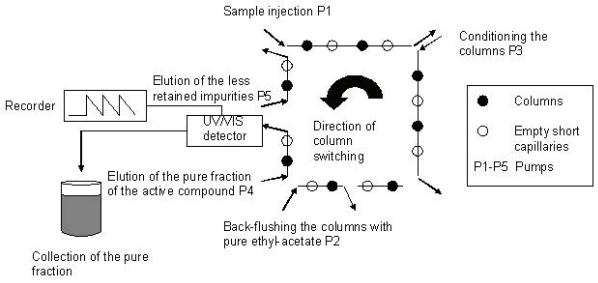

Fig. 3.: Quasi-continuous operation with the help of rotating switching valve of the SMB unit

The minimum number of columns required in the process is eight and in every second position an empty short capillary is inserted in the unit. One column and one empty position move together to the next position in every switching.

With the knowledge of the solvent volumes necessary to the subsequent steps of the separation, the switching time of the rotating SMB valve and flow rates of the pumps in the zones can be calculated.

The main steps of the elaboration of the quasi-continuous process are:

(A.) Preliminary elution experiment on one of the eight 250X16 mm I.D. columns used in the SMB unit for quasi-continuous elution separation, for the determination of the solvent volumes necessary to the steps of the separation. From these data the switching time of the rotating SMB valve and the pump flow rates can be calculated. (B.) Carrying out a quasi-continuous experiment applying the calculated parameters. (C.) Refining the process parameters in experimental way. (D.) Improvement the productivity by decreasing the switching time and proportionally increasing the flow rates of the pumps at the same time.

Experimental

The problem under investigation was the solution of quasi-continuous elution chromatographic purification of a steroid active compound, produced by Gedeon Richter Ltd. The starting point of the investigation was the pilot-plant batch elution chromatographic separation of the compound from its impurities, followed by a crystallisation step (not published here). This procedure has been applied successfully for years. The purity of the crude material was 94 % for the active compound. The goal was to produce the active compound with a purity of >99 %, the maximum level of individual impurities had to be under 0.3 %.

Chemicals

UETIKON C-490 15-35 μm Si gel was used as the stationary phase, it was purchased from ZEOCHEM AG (Switzerland).

The mobile phase was methylene chloride/ethyl acetate mixture. The back-flushing solvent was pure ethyl acetate. Solvents were purchased from Merck KgaA (Darmstadt, Germany).

Instrumentation

Preliminary Elution Experiment

The applied instrument consisted of a KNAUER K-501 HPLC pump, the volumetric flow rate was set to 5 mL min^{-1}, one of the eight 250X16 mm I.D. columns used in the SMB unit for quasi-continuous elution separation, packed with UETIKON C-490 15-35 μm Si gel packing material in our laboratory by dry-packing method using vibration, and a KNAUER UV/VIS filter photometer. The wavelength of the measurement was 254 nm. For the purpose of recording the chromatogram a Radelkis OH-850 potentiometric recorder was applied. The temperature was set to 25 ˚C.

Quasi-Continuous Experiments

A laboratory-scale SMB unit (KNAUER CSEP 9116) was used for the quasi-continuous elution experiments with five KNAUER K-501 HPLC pumps and eight columns. The columns were packed in our laboratory by dry-packing method, using vibration, as we described above. (Column dimension was 250X16 mm I.D.). The uniformity of columns was tested by elution investigations, injecting pure active compound on the columns and eluting it with the eluent. In the zone of the pure main fraction a KNAUER UV/VIS filter photometer was connected. The wavelength was set to 254 nm. For the purpose of recording the successive chromatograms a Radelkis OH-850 potentiometric recorder was applied. The temperature was set to 25 ˚C. (See *Fig. 4.*)

Fig. 4.: KNAUER CSEP 9116 SMB unit assembled for quasi-continuous process

Analytical Method Applied for the Quality Control of the Separation

The quality control of the purified active compound was performed by a validated analytical chromatographic method, which is applied for the time being in the current manufacturing process of the product.

Preliminary Elution Experiment

Before beginning the experiment, the column was equilibrated with the eluent. The injected sample amount was 0.32 g of crude material, which was solved in 5 ml methylene-chloride. After the injection step, the elution of less retained impurities was started and seven fractions were collected, for the purpose of determination of the first cut point of the pure main fraction. The end cut point of the peak was not so critical, because a certain impurity of small amount, eluting in the tail of the active compound peak can be removed by the crystallization step, following the chromatography. That was the reason, why there was no fraction collection there.

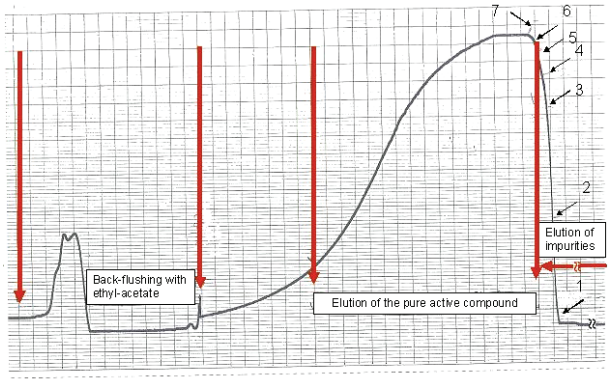

Fig. 5.: Chromatogram of the preliminary elution experiment

When the elution of the main compound was finished, the back-flushing with ethyl acetate was started and continued until a stable baseline was achieved. At the end of the back-flushing, the column conditioning was carried out with twice of the column volume of the eluent. Based on the pump flow rate and speed of the recorder, the solvent volumes, necessary to the subsequent steps of the separation could be calculated. *(Fig.5.)*

Quasi-Continuous Elution Experiments

The switching time of the rotating SMB valve was calculated based on the critical data of the preliminary elution experiment, such as the elution volumes of less retained impurities and the pure active compound. (See *Table 1.*) The pump flow rates were set in accordance with the calculated switching time and the solvent volumes, necessary to the steps of the separation.

Table 1: Results of the preliminary elution experiment

Steps of the separation	Solvent volumes necessary to the subsequent steps of the separation (mL)
Elution of less retained impurities	≈105
Elution of the pure active compound	≈84
Back-flushing with ethyl acetate	minimum 65
Column conditioning	twice of the column volume, minimum 100

The crude sample was solved in methylene-chloride, which was the weaker solvent component of the eluent. The compounds were adsorbed on the surface of packing material from this solution in a narrow band at the beginning of the columns, therefore the injected sample volume could be higher, in most cases exactly 10.5 mL. In this case the sample pump (P1) flow rate was set to 0.5 mL min^{-1}, which was a reasonable value.

In the quasi-continuous experiments the sample load was the same as in the preliminary elution experiment, namely 0.32 g of crude material. The exception was only the second experiment, in which the sample load was lower, exactly 0.29 g of crude material. The process parameters of the experiments are summarized in *Table 2.*

Table 2: Process parameters of quasi-continuous elution experiments

Experiment	Switching time (min)	Flow rates of the pumps (mL min^{-1})				
		Sample injection P1	Elution of less retained impurities P5	Elution of the pure active compound P4	Back-flushing P2	Column conditioning P3
1.	21	0.5	5	4	4	3
2.	19	0.5	5	4	4	3
3.	21	0.5	4.7	4	4	3
4.	21	0.5	4.5	2.5	4	3
5.	21	0.5	4.5	3.2	4	3
6.	21	0.5	4.5	3.5	4	3
7.	**21**	**0.5**	**4.5**	**3.5**	**4**	**3**
8.	**14**	**0.75**	**6.75**	**5.2**	**6**	**4.5**

Results and Discussion

Results of the Preliminary Elution Experiment

The aim of this experiment was to determine the solvent volumes necessary to the steps of the separation. The received data can be seen in Table 1.

Elaboration and Intensification of the Quasi-Continuous Process

As we mentioned before, based on the critical data of the preliminary elution experiment, such as the elution volumes of less retained impurities and the pure active compound, (Table 1.) the applied switching time was 21 min in the first experiment. In this first run the purity was very good, but the recovery was poor, because a significant part of the main fraction eluted in the zone of the less retained impurities. The reason of the deviation between the preliminary elution experiment and the first quasi-continuous run was the difference of the extra column volumes of the two systems in which the experiments were carried out. To improve the result of the first quasi-continuous run, two possible ways were tried:

The first was the reduction of the switching time, but in this case the sample load and volumes of solvents in each zone were also decreased, therefore finding the optimal set of process parameters was difficult. (2. experiment in Table 2.)

- The other (easier) way was decreasing the flow rate in the zone of the less retained impurities. (3. experiment)

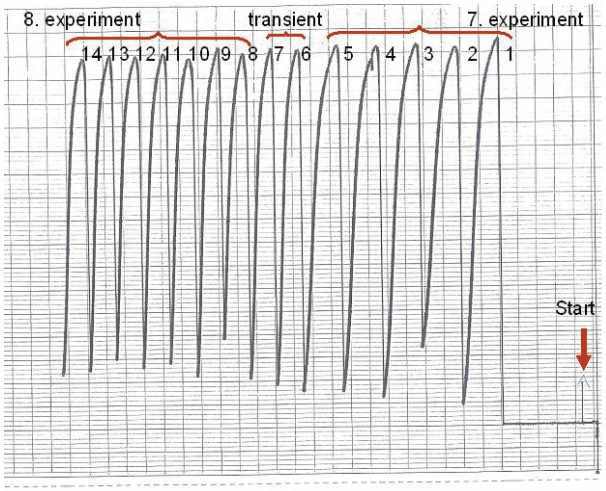

Fig. 6.: Chromatograms of the pure main fractions eluted from successive columns

In the 4., 5. and 6. experiments the cut point at the end tail of the main fraction was optimized with varying the flow rate in the zone of the active compound for the purpose of keeping the recovery as high as possible and the level of the above-mentioned impurity under a certain limit, with respect to the cleaning effect of the crystallisation step.

With the best process parameters a repetition was carried out. (7. experiment)

In the final step (8. experiment) the productivity was increased with decreasing the switching time and proportionally increasing the flow rates at the same time. The last two runs, switching from parameter set of 7. experiment to the ones of 8. experiment, with two transient peaks can be seen in *Fig. 6.*

The reason of transients is that, when the experimental parameters were changed, the peak of the main fraction from the actually injected sample eluted after two switching. That is why the new valuable status was possible to see on the recorder after two transient peaks.

The specific capacity parameters of 7. and 8. runs, in comparison with the pilot-plant batch elution separation are shown in *Table 3.*

Table 3: The specific capacity parameters of 7. and 8. runs in comparison with the pilot-plant batch elution separation

Experiment	Specific capacity parameters			
	Specific solvent consumption Methylene chloride (mL g^{-1})	Specific solvent consumption Ethyl acetate (mL g^{-1})	Productivity [g (kg packing hour)$^{-1}$]	Recovery (m m^{-1} %)
Batch elution pilot-scale	499.51	214.34	10.15	84.26
7.	836.02	409.97	4.11	86.85
8.	761.58	374.30	6.75	95.06

In the 8. experiment a higher recovery resulted a little bit higher level of impurities in the product, but the quality still met the requirements.

Conclusion

The quasi-continuous elution chromatographic purification of a steroid active compound was possible to carry out successfully with the help of the rotating switching valve of a laboratory-scale Simulated Moving Bed (SMB) instrument. The quality of the product, gained at optimum operating conditions met the purity requirements (purity > 99 *%*, maximum level of individual impurities < 0.3 *%)*.

The main advantage of applying this quasi-continuous separation method is that the staff need for processing is less, than in case of batch elution chromatographic separation. Fraction collector is not needed, because cutting is done automatically by switching the columns and the main fractions coming from successive columns can be pooled in one vessel.

Comparing the specific capacity parameters of the quasi-continuous process with the batch pilot-scale separation, the specific solvent consumption is higher and the productivity is lower. But mention must be made, that the number of columns applied in the SMB instrument were eight instead of the necessary five because of the construction of the SMB unit. The columns applied in the SMB instrument were shorter than the pilot-scale column, so the theoretical plate number of these columns was considerably lower, therefore the specific sample load also had to be lower. (In case of pilot-scale column the specific sample load was 0.02 [g sample g^{-1} packing material], while in quasi-continuous process it was only 0.0143 [g sample g^{-1} packing material].)

The robustness of the process demands the uniformity of columns inserted in the SMB instrument.

The change of temperature has strong impact on the adsorption behaviour of compounds, therefore transients in temperature can detrimentally influence the separation.

SYMBOLS

I.D.	Internal diameter
F	Feed
c	Concentration of the compounds
R	Raffinate
E	Extract
L	Liquid recycle
D	Fresh solvent
P	“Recycle of the packing”, column switching time

INDICES

W	Less retained compound
S	More retained compound
F	Feed
R	Raffinate
E	Extract

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