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Purification of S-PG05 using Simulated Moving Bed Chromatography

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Abstract: S-PG05 ((15S)-15-methyl-prostaglandin $F_2\alpha$ methyl ester) was separated from the epimer R-PG05 and other impurities using SMB, combined with batch chromatography. Based on this technical procedure, 94% pure S-PG05 was obtained. Different column configurations and separation conditions of SMB have been selected. A non-synchronous shift with a "partial extract withdrawal" strategy has been employed, resulting in the saving of desorbent and the increase of the purity of S-PG05.

Keywords: Non-synchronous shift, prostaglandins, partial extract withdrawal, S-PG05, simulated moving bed Chromatography

INTRODUCTION

15-methyl-prostaglandin $F_2\alpha$ methyl ester (PG05) belongs to the class prostaglandins. Its chemical structure is shown in Fig. 1. Prostaglandins are very important compounds for humans since they possess comprehensive and very strong bioactivity (1–4). Prostaglandins can be obtained from natural products (5) or via chemosynthesis (6–8). The manufacture of PG05 discussed in this paper is accomplished with the method of chemosynthesis (9). And the product of chemosynthesis is composed of epimers (S-PG05 and R-PG05) and many impurities. For pharmaceutical

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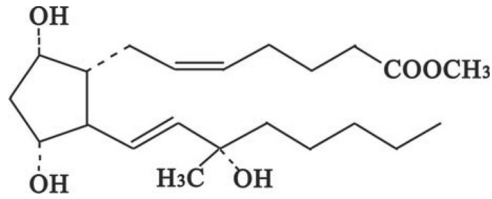

Figure 1. The chemical structure of PG05.

applications, only the S-PG05 (trade name Carboprost Methyl) possesses the biological activity which is used for the selective termination of gestation (10–15).

Originally the purification of S-PG05 is employed via the batch chromatography (50 mm × 300 mm I.D), where the packing material is silicon gel and the desorbent is a mixture of CH_2Cl_2 (dichloromethane) and CH_3COCH_3 (acetone). In this process, the consumption of silicon gel and the mobile phase are much larger, since the silicon gel must be changed after each elution. On the other hand, since the batch process is intermittent, so the efficiency is lower. Considering the factors such as scale, the consumption, the efficiency, and environmental protection, the reversed-phase SMB chromatography is used where silicon gel is replaced with C_{18} packing material and the dichloromethane and acetone is replaced with water and methanol, since C_{18} packing material can be used about one season and the methanol can be recovered in 70%. So the total cost and the pollution degree are reduced and the efficiency is raised.

This paper describes the purification of S-PG05 using a two-step chromatographic process, consisting of a pre-treatment step of batch chromatography followed by the 3-zone simulated moving bed running under a non-synchronous shift with a "partial extract withdrawal" mode. The purity of S-PG05 obtained via synthesis is only 15%. Therefore SMB is combined with preparative chromatography as the pre-treatment. The preparative chromatography is mainly used to remove the majority of the impurities; the SMB process is used to separate the target component S-PG05 from the epimer R-PG05 and the other remaining impurities.

To raise the purity of S-PG05 and reduce the consumption of the desorbent in the SMB process, a non-synchronous shift with a "partial extract withdrawal" strategy is employed in this work. It is not same with the VARICOL process that has been described in (16–19). The principle of the VARICOL process is also based on a non-synchronous shift of the inlet and outlet valves in a multicolumn system, in contrast to the SMB operation where this shift is synchronous. But in standard VARICOL operation the flow rates are kept constant. In this paper, during half of

the switching interval the extract withdrawal port is closed and the desorbent flow rate is reduced, so we call it a non-synchronous shift with a "partial extract withdrawal" strategy.

EXPERIMENTAL

Materials

The synthesis product and the standard sample of S-PG05 were provided by Northeast General Pharmaceutical Factory (Liaoning, P. R. China). HPLC-grade methanol used in the analysis was purchased from Yuwang Industrial Co., Ltd (Shandong, P. R. China). The methanol used in industrial tests was made by Northeast General Pharmaceutical Factory. The tracers, uracil and naphthalene, which were used to measure the porosity and the column efficiency, were purchased from Shenyang reagent factory (Liaoning, P. R. China) and Shanghai hengxin chemical reagent Co., Ltd (Shanghai, P. R. China), respectively.

Sample Analysis

A Waters HPLC system was used in analytical experiments. It consists of a 515 HPLC pump, a Rheodyne 7725i injector and a 2487 UV detector. A 150 mm × 4.6 mm I.D analytical column (Zorbax SB-C_{18}, Agilent) was chosen.

The mobile phase was a mixture of methanol and water, (7:3 V/V). The flow rate was 1.0 mL/min. The detector wavelength was 202 nm. The purity of S-PG05 was determined by an external reference method.

Pretreatment by Batch Chromatography

In the synthesis product, besides the target component S-PG05, the epimer R-PG05 and other impurities are also present. The chromatogram of the crude synthesis product shows that the retention time of S-PG05 is larger than that of R-PG05, and that of some of the impurities are eluted before R-PG05 (less retained impurities) and that of other impurities are eluted after S-PG05 (more retained impurities). Therefore, the presence of these impurities would seriously disturb the separation and purification of S-PG05 in the SMB process. So a pretreatment process by batch chromatography to remove the majority of impurities before SMB step is necessary.

A 100 mm × 100 mm I.D column (short column) linked with a 260 mm × 100 mm I.D column (long column) was used in this pretreatment process. The column was packed with spherical packing material C_{18} in 30–40 μm particle size (The Great Eur-Asia Sci and Tech development Co., Ltd. China). The sample concentration was 0.5 g/ml (the solvent was 70% methanol (v/v)) and the quantity injected was 100 g each time. A solution with 50% methanol (v/v) was used as the mobile phase to elute from the short column to the long column for 8 hours at a flow rate of 75 ml/min. Then the mobile phase was replaced by a solution with 70% methanol (v/v). When more retained impurities were eluted from the outlet of the short column to the inlet of the long column, the short column was removed and cleaned by pure methanol while the long column still was eluted with a solution with 70% methanol (v/v) until all S-PG05 were desorbed. The process was operated at a flow rate of 75 ml/min. The fractions which contain more S-PG05 (about 40%) were collected and dried via the rotary evaporators. The product of pretreatment was subsequently purified using SMB.

SMB Experiments

SMB System

The three-zone SMB used in this test is designed and set up by our laboratory. It consists of eight dynamic axial compression columns (120 mm × 50 mm I.D) which packed with the C_{18} packing material (The Great Eur-Asia Sci and Tech Development Co., Ltd., China). And two constant-flow pumps (Beijing Xingda Technology Co., China) are used to inject feed and desorbent. The flow rates in the two outlets (extract and raffinate) are controlled by two flow controllers (Yuyao Kingtai Instrument Co., China). A solenoid valve (END-Armaturen GmbH & Co. KG, Germany) is attached at the inlet and at the outlet of each column and at the joint points of columns; a check valve is connected after each solenoid valve. The schematic diagram of this SMB set-up is shown in Fig. 2.

Selection of Column Configuration and Measurement of Internal Concentration Profiles

After the SMB operation conditions were selected, the SMB experiments were employed with different column configuration (1/4/3(run1) and 1/3/4(run2)). A configuration of 1/4/3 in a 8-column system represents

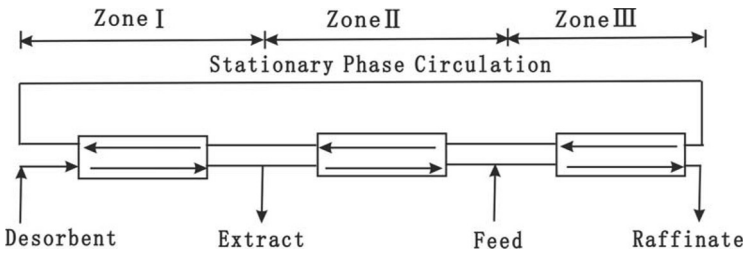

Figure 2. Schematic diagram of a three-zone SMB.

a system which zone I always contains one column, zone II always contains four columns, and zone III always contains three columns. When the SMB system reached steady state, the internal concentration profiles were measured. The internal concentration profiles were determined by off-line analysis. And the samples were taken from the outlet of each column at the end of the switch period.

A Non-Synchronous Shift with a "Partial Extract Withdrawal" Strategy

When the SMB with optimum column configuration (1/3/4) reached steady state, a non-synchronous shift with a "partial extract withdrawal" strategy was employed. A non-synchronous shift with a "partial extract withdrawal" process is shown in Fig. 3. As can be seen from Fig. 3, the initial column configuration is characterized by one column in zone

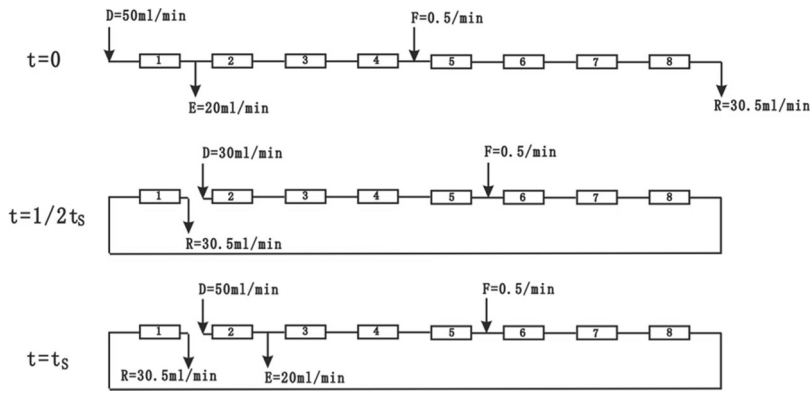

Figure 3. The non-synchronous shift with a "partial extract withdrawal" operation.

Table 1. The number of columns in each zone of the non-synchronous shift with a "partial extract withdrawal" operation

		Number of columns		
Fraction of the period	Duration (min)	I	II	III
$0 \sim 1/2t_S$	17.5	1	3	4
$1/2t_S \sim t_S$	17.5	0	4	4
Average number of columns		0.5	3.5	4

I, three columns in zone II, and four columns in zone III during the first half period (from 0 to $1/2t_S$). At $1/2t_S$, the desorbent, feed, and raffinate lines shift simultaneously, whereas the extract line does not shift and the extract withdrawal port is closed. The column configuration becomes 0:4:4 and the desorbent flow rate changed from 50 ml/min to 30 ml/min. At the end of the period, the extract line is shifted and the extract withdrawal port is opened again; the other lines do not shift, so the column configuration resumes 1:3:4. This time pattern is repeated the same in the next period. The average number of columns for three zones is 0.5:3.5:4, respectively (in Table 1). During the whole process, the flow rate of feed and the raffinate are 0.5 ml/min and 30.5 ml/min.

FITTING OF THE ISOTHERM PARAMETERS AND SELECTION OF THE OPERATION CONDITIONS OF SMB

Fitting of the Isotherm Parameters

The competitive isotherms were determined with the inverse method (20,21).

The competitive Langmuir isotherm for each component is defined as:

$$q_i = \frac{q_S b_i c_i}{1 + b_A c_A + b_B c_B} = \frac{G_i c_i}{1 + b_A c_A + b_B c_B} \quad i = A, B$$

where q_S and b are the saturation concentration in the stationary phase and the adsorption equilibrium constant, respectively. G is the adsorption coefficient. Subscript A denotes the main retained component, S-PG05; subscript B denotes the less retained component, R-PG05.

Table 2. Parameters of each column in SMB

Serial number	t_m/min	ε
1	7.55	0.64
2	7.40	0.63
3	7.60	0.65
4	7.50	0.64
5	7.50	0.64
6	7.42	0.63
7	7.45	0.63
8	7.67	0.65
Average values	7.51	0.64

For convenience, the determination of the isotherm was made on the semi-preparative column (150 mm × 10 mm I.D) packed with the same material in the SMB columns. The porosity of this column has the same value (0.64) as the average porosity of the columns in the SMB (in Table 2). The void times of each column listed in Table 2 were measured at a flow rate of 20 ml/min.

A low concentration (0.0025 g/ml) injection of the epimers was used to determine the parameters of the Langmuir isotherm G_A and G_B. A higher concentration (0.05 g/ml) injection of the epimers was used to obtain the band profile. Through the fitting between the experimental profile and a series of numerical profiles based on the inverse method (see Fig. 4), the values of b_i (parameters of isotherm of epimer mixture) were determined and are listed in Table 3.

Determination of the Complete Separation Region

According to the "triangle method" (22,23), the complete separation region in the (m_2, m_3) plane was obtained by inserting the isotherm parameters and $c_A = 0.03$ g/ml, $c_B = 0.02$ g/ml into the correlative equations (22). The separation region is given by the triangle "bwr" which is depicted in Fig. 5.

Selection of the Operation Conditions of SMB

From the triangle in Fig. 5 and the Van-Deemter curve, the point A (see Fig. 5) was chosen as the operation point at which the flow rate of desorbent and the extract are 50 ml/min and 20 ml/min (corresponding to the flow rate of 1.61 cm/min in Fig. 6). And the flow rate of the feed is 0.5 ml/min and the switch time is 35 min.

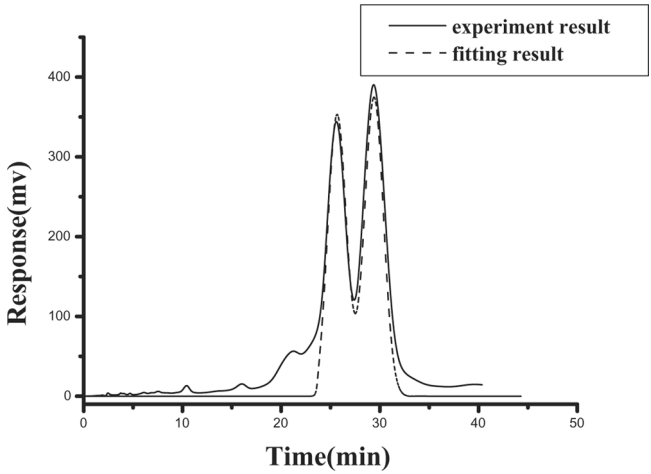

Figure 4. Comparison of the numerical result and experiment result. Dotted line is experiment result, symbol is numerical result.

RESULTS AND DISCUSSION

Pretreatment Process

In the pretreatment process, the majority of the impurities and a part of R-PG05 were removed. The purity of the target component S-PG05 was raised from 15% to 40%. The chromatograms of the chemosynthesis product and the product of pretreatment process are compared in Figs. 7a, 7b.

SMB Process

The Effect of Column Configuration on Separation

The operation conditions (point A in Fig. 5) and the separation results of the experimental runs at the end of the period are shown in Table 4. The internal concentration profile for run2 is shown in Fig. 8.

Table 3. Isotherm parameters

	G	b/(ml/g)
S-PG05 (component A)	12.20	0.89
R-PG05 (component B)	10.42	0.76

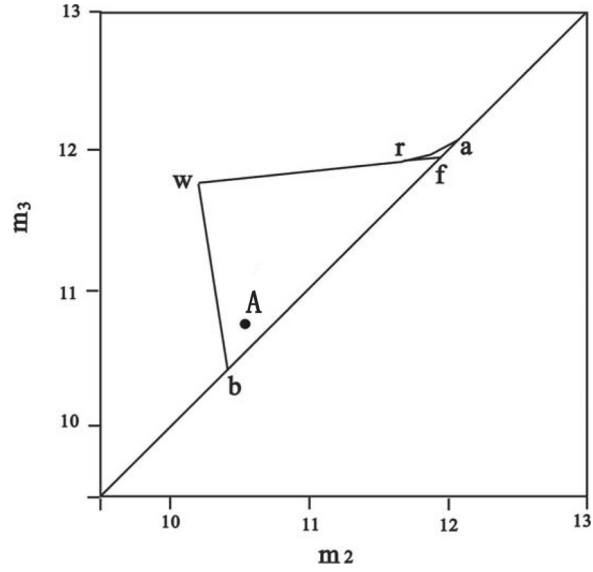

Figure 5. (m_2-m_3) plane.

The results in Table 4 show that the separation result in run2 is better than that in run1. It is due to the number of columns in zone II is more one in run1 than that in run2. The flowing distance of S-PG05 before it is extracted from the outlet is more in run1 than that in run2. In run1, the

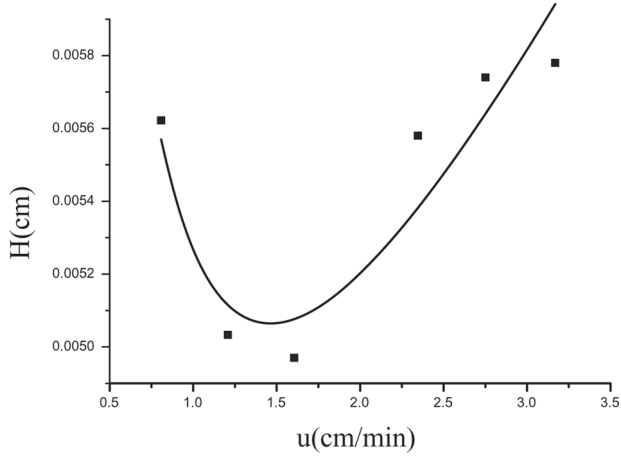

Figure 6. Van-Deemter curve measured in 1st column.

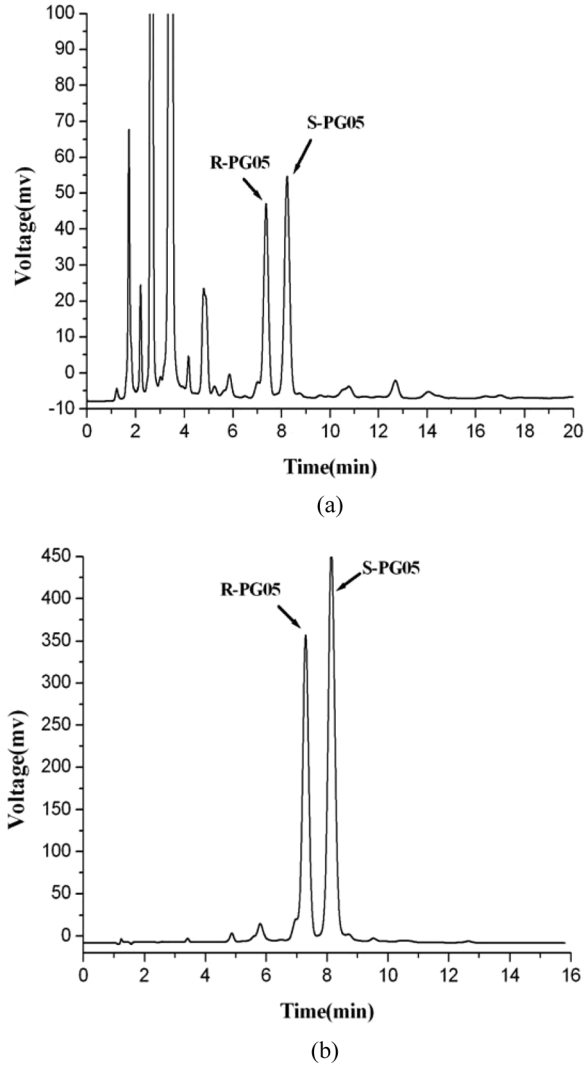

Figure 7. Chromatograms of (a) the chemosynthesis product, (b) the product after pretreatment (i.e., feed of SMB).

concentration of a more retained component (S-PG05) and a less retained component (R-PG05) are high at the end of zone III and the recovery of S-PG05 reduced. In run2, the internal concentration profile for run2 is normal and the S-PG05 was separated well from the mixture which purity was 92%.

Table 4. Operation conditions and separation results of experimental runs

Run	Column configuration	Feed concentration (g/ml)	Flow rate/(ml/min)			Switch time (min)	Experimental purity values	
			Desorbent	Extract	Feed		P_E (%)	P_R (%)
1	1/4/3	0.05	50	20	0.5	35	85	78
2	1/3/4	0.05	50	20	0.5	35	92	93

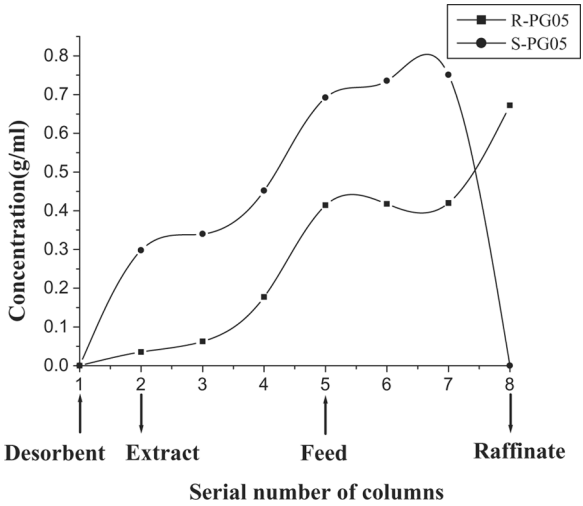

Figure 8. Internal concentration profile in run2 at the end of the switch period.

A Non-Synchronous Shift with a "Partial Extract Withdrawal" Strategy

In order to improve the purity of S-PG05, a non-synchronous shift strategy was employed, so the number of columns in zone II was increased by using 3.5 columns; on the other hand, through this switch, the more retained impurities were "moved" from zone I to zone III and were eluted from the raffinate outlet. The purity of S-PG05 collected at the extract outlet increased from 92% to 94%.

Here, the "partial extract withdrawal" process was also employed, during half of the switching interval the extract withdrawal port was closed and the desorbent flow rate decreased from 50 ml/min to 30 ml/min. So the desorbent was reduced by 21.2%. If the number of columns in zone I is zero in the non-synchronous shift process, the desorbent can save by the partial extract withdrawal operation.

This process can save the desorbent and increase the purity comparing to common SMB. This process is similar to Varicol but not the same as the Varicol process. For the standard Varicol operation, the flow rates are kept constant in all processes. The operation conditions of the standard Varicol process are not the same as the SMB that included the same columns and it needs to be optimized again. But the flow rates of zone II and zone III are the same as the SMB operation in this work. So the optimization of the operation conditions is easy, the operation conditions of the SMB included the same columns can be used directly.

CONCLUSION

The results show that it is feasible to separate and purify the epimer S-PG05 from a chemosynthesis product by 3-zone simulated moving bed running under a non-synchronous shift with a "partial extract withdrawal" mode, consisting of a pre-treatment step of batch chromatography.

The non-synchronous shift with a "partial extract withdrawal" operation helps to save the desorbent and improve the purity of the target component S-PG05, especially, compared to a three-zone SMB since the consumption of the desorbent is larger.

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NOTATION

q_i	concentration of component i in the stationary phase (g/ml)
c_i	concentration of component i in the mobile phase (g/ml)
q_S	saturation concentration in stationary phase (g/ml)
b	adsorption equilibrium constant (ml/g)
G	adsorption coefficient
m_j	mass flow-rate ratio in zone j
H	height equivalent to a theoretical plate (cm)
c_A	initial concentration of S-PG05 (g/ml)
c_B	initial concentration of R-PG05 (g/ml)
u	linear velocity (cm/min)
t_m	dead time (min)
t_R	retention time of the component (min)
t_S	switch time (min)
ε	total porosity
S-PG05	(15S)-15-methyl-prostaglandin $F_2\alpha$ methyl ester
R-PG05	(15R)-15-methyl-prostaglandin $F_2\alpha$ methyl ester

D	desorbent
E	extract
F	feed
R	raffinate

Subscript

A	more retained component of the feed
B	less retained component of the feed

REFERENCES

1. Igarashi, R.; Takenaga, M.; Takeuchi, J.; Kitagawa, A.; Matsunoto, K.; Mizushima, Y. (2001) Marked hypotensive and blood flow-increasing effects of a new lipo-PGE_1 (lipo-AS013) due to vascular wall targeting. *J Control Release.*, *71* (2): 157–164.
2. Straus, D.S.; Glass, C.K. (2001) Cyclopentenone prostaglandins: New insights on biological activities and cellular targets. *Med Res Rev.*, *21* (3): 185–210.
3. Zou, Y.H.; Chiou, G.C.Y. (2005) New ideas for medical therapy of glaucoma in the future. *International Journal of Ophthalmology*, *5* (3): 411–422.
4. Olander, K.; Zimmerman, T.J.; Downes, N.; Xalacom, J.S. (2004) Switching from latanoprost to fixed-combination latanoprost-timolol: a 21-day, randomized, double-masked, active-control study in patients with glaucoma and ocular hypertension. *Clinical Therapeutics*, *26* (10): 1619–1629.
5. Bergstrom, S.; Ryhage, R.; Sjovall, J. (1960) The isolation of prostaglandin E1 from sheep prostateglands. *Acta Chem Scand*, *14*: 1701–1705.
6. Fujimori, K.; Inui, T.; Uodome, N.; Kadoyama, K.; Aritake, K.; Urade, Y. (2006) Zebrafish and chicken lipocalin-type prostaglandin D synthase homologues: Conservation of mammalian gene structure and binding ability for lipophilic molecules, and difference in expression profile and enzyme activity. *Gene*, *375*: 14–25.
7. Zhao, Z.; Araldi, G.L.; Xiao, Y.; Reddy, A.P.; Liao, Y.; Karra, S.; Brugger, N.; Fischer, D.; Palmer, E. (2007) Synthesis and evaluation of novel pyrazolidinone analogs of PGE_2 as EP_2 and EP_4 receptors agonists. *Bioorganic & Medicinal Chemistry*, *17* (23): 6572–6575.
8. Fernández-Dueñas, V.; Sánchez, S.; Planas, E.; Poveda, R. (2008) Adjuvant effect of caffeine on acetylsalicylic acid anti-nociception: Prostaglandin E2 synthesis determination in carrageenan-induced peripheral inflammation in rat. *European Journal of Pain*, *12* (2): 157–163.
9. Yuanliu, W.; Guangzhong, Y.; Biyan, J.; Chiayu, H.; Zhirong, W. (1981) Synthesis of d,1-15-methyl prostaglandin $F_{2\alpha}$ methyl ester and its 15-epimer. *Acta academic medicine sinicae*, *3* (2): 136–137.
10. Marrs, R.P.; Brenner, P.F.; Roy, S.; Daniel, R.; Mishell, Jr. (1981) Termination of early gestation with (15S)-15-methyl prostaglandin $F_{2\alpha}$ methyl ester vaginal suppositories. *Contraception*, *24* (6): 617–630.

11. Wilks, J.W. (1980) Effects of (15S)-15-methyl prostaglandin $F_{2\alpha}$ methyl ester and estrogens upon the corpus luteum and conceptus of the rhesus monkey. *Prostaglandins*, *20* (5): 807–823.
12. Scher, J.; Dai-Yun, J.; Thomas, D.; Kerenyi. (1980) Post-conceptional induction of menses with a single vaginal suppository of (15S)-15-methyl prostaglandin $F_2\alpha$ methyl ester. *Prostaglandins*, *20* (3): 469–479.
13. Roux, J.F.; Southern, E.; Gutknecht, G. (1980) The effect of a 15(S)-15-methyl prostaglandin $F_{2\alpha}$ (methyl ester) suppository upon termination of early pregnancy. *Contraception*, *22* (1): 57–61.
14. Roy, S.; Brenner, P.F.; Marrs, R.P.; Mishell, D.R. (1980) Termination of early gestation with a single vaginal suppository of (15s)-15-methyl-prostaglandin $F_2\alpha$ methyl ester. *Contraception*, *22* (2): 137–152.
15. Duenhoelter, J.H.; Ramos, R.S.; Milewich, L.; Macdonald, P.C. (1978) Interruption of early pregnancy with a silastic device containing (15S)-15-methyl prostaglandin $F_{2\alpha}$ methyl ester: Efficacy and mode of action. *Contraception*, *17* (1): 51–60.
16. Ludemann-Homburger, O.; Nicoud, R.M.; Bailly, M. (2000) The VARICOL process: a new multicolumn continuous chromatographic process. *Sep.Sci. Technol.*, *35* (12): 1829–1862.
17. Adam, P.; Nicoud, R.M.; Bailly, M.; Ludemann-Hombourger, O. (2000) Process and device for separation with variable length. U.S. Patent 6,136,198.
18. Ludemann-Homburger, O.; Pigorini, G.; Nicoud, R.M.; Ross, D.S.; Terfloth, G. (2002) Application of the "VARICOL" process to the separation of the isomers of the SB-553261 racemate. *J Chromatogr A.*, *947*: 59–68.
19. Pais, L.S.; Rodrigues, A.E. (2003) Design of simulated moving bed and Varicol processes for preparative separation with a low number of columns. *J. Chromatogr A.*, *1006*: 33–44.
20. Felinger, A.; Cavazzini, A.; Guiochon, G. (2003) Numerical determination of the competitive isotherm of enantiomers. *J. Chromatography A.*, *986* (2): 207–225.
21. Cong, J.; Lin, B. (2007) Separation of Liquiritin by simulated moving bed chromatography. *J Chromatography A.*, *1145* (1–2): 190–194.
22. Mazzotti, M.; Storti, G.; Morbidelli, M. (1997) Optimal operation of simulated moving bed units for nonlinear chromatographic separations. *J. Chromatography A.*, *769* (1): 3–24.
23. Migliorini, C.; Mazzotti, M.; Morbidelli, M. (1998) Continuous chromatographic separation through simulated moving beds under linear and nonlinear conditions. *J. Chromatogr A.*, *827* (2): 161–173.