

Method Development for Chiral Purification of Racemic Clopidogrel Using Chiral Stationary Phase

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

HPLC method was developed for resolution of racemic clopidogrel into its chirally pure enantiomer of interest. Screening experiments were performed using amycoat and cellucoat analytical chiral stationary phases in organic solvent based polar and nonpolar eluents in various combinations. Based on the screening outcomes, the compound loading studies were carried out using amycoat higher μ particle sized chiral stationary column by keeping in mind the scale up studies to avoid high back pressure that can limit the efficiency of separation.

Keywords: Clopidogrel; chromatography; enantiomer; preparative HPLC.

1. INTRODUCTION

The study and pharmaceutical development of individual enantiomers and racemates usually

require specialized chiral techniques for their accurate identification, characterization, separation and measurement. They are often readily distinguished by biological systems, and

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possibly will have different pharmacokinetic properties like absorption, distribution, biotransformation and excretion moreover quantitatively or qualitatively different pharmacologic or toxicologic effects [1].

When stereoisomers are biologically distinguishable, they behave as different drugs, though it has been past practice to develop racemates. The properties of the individual enantiomers have not generally been well studied or characterized. Whether separated enantiomers should be developed was largely a theoretical question for the intention that commercial separation of racemates was challenging. Nowadays that technological developments like large scale chiral separation procedures or asymmetric syntheses permit the fabrication of many single enantiomers on a commercially viable scale, it is appropriate to consider what FDA's strategy with respect to stereoisomeric mixtures should be. Development of racemates raises issues of acceptable manufacturing control of synthesis and impurities, adequate pharmacologic and toxicologic assessment, proper interpretation of metabolism and distribution, and appropriate clinical evaluation [2-3].

Where little difference is observed in activity and disposition of the enantiomers, racemates may be developed. In some situations, development of a single enantiomer is particularly required where one enantiomer has a toxic or undesirable pharmacologic effects and the other does not have. Therefore measurement and control of enantiomeric purity of chiral active pharmaceutical ingredients (APIs) is a necessary means to control the quality of drug substances as only one enantiomer has desired biological and pharmacological properties. Therefore, chiral drugs must be chirally pure, which places great demands on their synthesis,[4-5] analysis and purification [6-9]. Development of chirally pure drugs relies on four fundamental technologies: asymmetric synthesis [10], chiral resolution via crystallization [11-12] or diastereomeric salts,[13-14] enantiomeric separation on chiral stationary phase (CSP) [15] and biocatalytic or enzymatic synthesis [16]. Asymmetric synthesis is usually expensive and relatively challenging. Biocatalytic or enzymatic resolution is an attractive substitute if the enzyme of the interest is commercially accessible. Chiral resolution via crystallization is a widely used technique for manufacture of chirally pure drugs and chiral chromatography has become a preferred method for continuous separation of enantiopure compounds especially

where the use of chiral catalysts are limited due to IP issues (Intellectual property rights: Patents, copyrights etc.); both these methods are commercially feasible if the undesired enantiomer is reprocessed. Preparative batch chromatography is being replaced with continuous chromatographic processes like simulated moving bed (SMB) [17].

Enantioselective chromatographic separation can also be carried out on achiral chromatographic columns using a chiral mobile phase or a chiral additive [18]. In our continued efforts to develop HPLC methods for separation of enantiomers of key racemic APIs at pilot scale like preparative batch chromatography and continuous separation processes, SMB/Varicol separation. We have considered racemic clopidogrel in this work and will present outcome of these investigations for the API.

Most of the enantioseparation demands for pharmaceutical development can be met with traditional elution chromatography approaches utilizing 'touching band' separations (i.e. separations in which the two enantiomers are still baseline or nearly baseline resolved). Moving from the touching band situation to the sample overload situation results in a complex peak, where peaks become merged to a degree. Chromatographic productivity using such an approach can be superior to the touching band method, provided one has investigated the appropriate place for fraction cutting within the complex chromatographic peak. In addition, the solvent requirements for such separations can be reduced, although a considerable disadvantage is the need to collect and reprocess a middle fraction consisting of a mixture of the two peaks. The technique of simulated moving bed chromatography, by using multiple columns, can allow continuous separation with uninterrupted reprocessing of the mixed fraction. SMB chromatography has recently gained attention as a useful tool for industrial-scale chromatographic separations [19-22] and can often lead to gains in productivity and reduced solvent consumption, which can improve the economics of a separation process at the appropriate scale [23]. A single column innovation has been developed [24] that utilizes recycling of the mixed fraction with augmentation with fresh feed solution so that a 'steady-state recycling' situation can be attained.

It should be noted that the increase in productivity and the solvent savings of SMB chromatography relative to touching band elution

chromatography is most dramatic for those separations having poor chromatographic selectivity. The 'SMB advantage' is much reduced for the most enantioselective and highly productive separations. As a general rule, highly productive chromatography requires either highly selective separation media or high performance separation equipment, but not both.

1.1 Summary

The purification of the racemic clopidogrel was successfully performed on a Kromasil 10 μm AmyCoat column with heptane/IPA (Isopropyl alcohol) 98/2 (v/v), as well as 100% IPA as mobile phase. The heptane based mobile phase rendered better selectivity ($\alpha=1.36$) compared to 100% IPA ($\alpha=1.28$), however the racemate only dissolved at concentration 15 mg/mL in heptane/IPA 98/2. For batch chromatography a productivity of $26 \text{ g}_{\text{racemate}} \times \text{kg}_{\text{CSP}}^{-1} \times \text{h}^{-1}$ was obtained in pure IPA, up to 50 mg/mL could be dissolved, which offers a distinct advantage in preparative HPLC, and moreover in SMB-type of purification. The key results are displayed in Table 3.

As can be seen in the results (Table 1), 100% IPA is only an interesting option for SMB type separation due to the poor recovery for a pure 2nd enantiomer. A single solvent offers however advantages with respect to ease of handling, e.g.

recycling. For SMB type separations, larger particle sized CSP is generally used to avoid high back pressure in columns connected in series.

2. EXPERIMENTAL

All the APIs (active pharmaceutical ingredients) and the API intermediates were provided by Ranbaxy Laboratories Ltd., India. Waters HPLC with PDA 2998 and RI 2410 detectors were used for method development. HPLC grade solvents were used as obtained from Rankem and Qualigens. Chiral columns were procured from Kromasil. Kromasil AmyCoat and CelluCoat chiral stationary phases were screened for highest selectivity using different mobile phases.

2.1 Experimental Conditions

Columns	: Kromasil AmyCoat and CelluCoat, 3 μm (4.6x150 mm)
Sample solution	: 1 mg racemate / mL mobile phase, 10 μL injection
Flow rate	: 1 mL/min or 2 mL/min
Temperature	: 20 $^{\circ}\text{C}$
Detection	: 254 nm

The results of the screening experiments are displayed in Table 2.

Table 1. Summary of purity and recovery results for the preparative separation of clopidogrel on AmyCoat 10 μm . The results are based on injecting 13.5 mg racemate onto a 4.6x250 mm column. Detailed experimental conditions are described in experimental section

Mobile phase	k'2	Selectivity α	Purity	Recovery [%]
Heptane/IPA 98/2	2.25	1.36	E1: 99.5% E2: 98.3%	E1: 83.0% E2: 69.2%
IPA	1.45	1.28	E1: 100% E2: 98.1%	E1: 28.4% E2: 16.9%

*IPA-Isopropyl alcohol

Table 2. Results from screening experiments

Stationary phase	Mobile phase	k'1	k'2	Selectivity α
AmyCoat 3 μm	Heptane/IPA 90/10	0.49	0.71	1.45
AmyCoat 3 μm	Heptane/IPA 95/5	1.01	1.36	1.35
AmyCoat 3 μm	Heptane/IPA 98/2	1.66	2.25	1.36
AmyCoat 3 μm	100% IPA	1.13	1.45	1.28
AmyCoat 3 μm	100% MeCN	Elutes with the void		
CelluCoat 3 μm	Heptane/IPA 98/2	1.47	1.59	1.08
CelluCoat 3 μm	Heptane/EtOH 98/2	0.92	0.95	1.03

*MeCN-Acetonitrile, EtOH-Ethanol

The analytical separation of racemic clopidogrel with AmyCoat using heptane/IPA 98/2 and 100% IPA are displayed in Fig. 1.

mobile phase on AmyCoat 10 μ m was performed. The overlaid chromatograms are shown in Fig. 2.

2.2 Overloading Study

2.3 Preparative Separations

Based on the results shown above, an overloading study using the heptane/IPA 98/2

In total, four preparative separations were performed with clopidogrel using AmyCoat 10 μ m as stationary phase. The experimental conditions are described in Table 3.

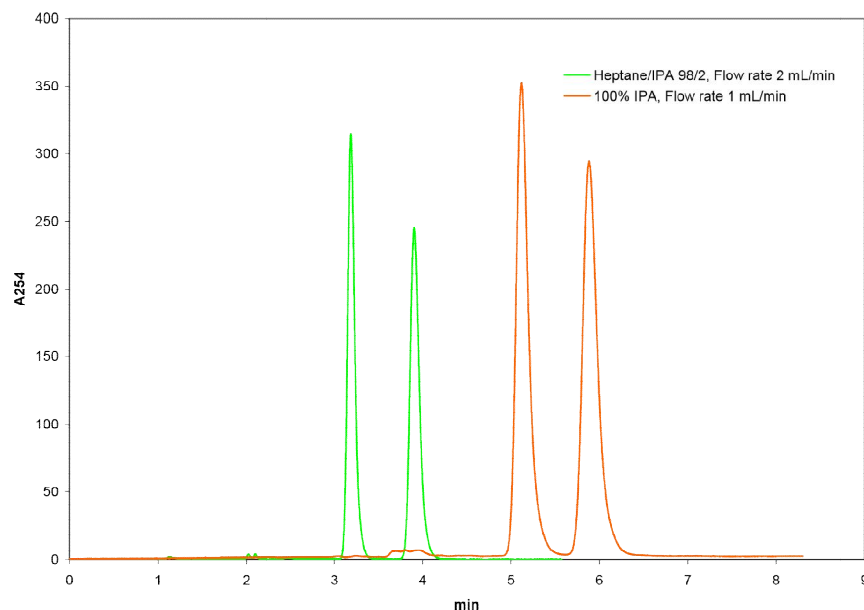


Fig. 1. Analytical chromatogram of clopidogrel with AmyCoat 3 μ m (4.6x150 mm). Different flow rates were used, due to higher pressure drop for 100% IPA

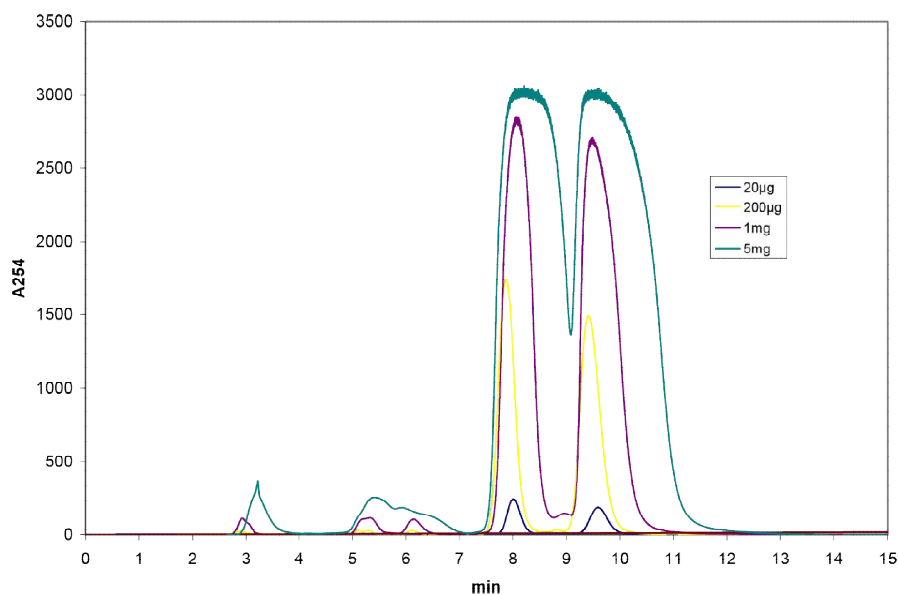


Fig. 2. Overloaded chromatograms of clopidogrel on Kromasil 10 μ m (4.6x250 mm)
 Mobile phase: Heptane/IPA 98/2. Flow rate: 1 mL/min, Sample solution: 10 mg/mL mobile phase

Table 3. Experimental conditions for the preparative separations of the racemic clopidogrel on Kromasil AmyCoat 10 µm (4.6x250 mm)

	Separation 1	Separation 2	Separation 3	Separation 4
Mobile phase	Hep/IPA 98/2 (v/v)	Hep/IPA 98/2 (v/v)	IPA	IPA
Feed solution	15 mg Clopidogrel/ mL mobile phase	15 mg Clopidogrel / mL mobile phase	50 mg Clopidogrel/ mL IPA	50 mg Clopidogrel/ mL IPA
Injection volume	533 µL	900 µL	270 µL	500 µL
Loading	8 mg	13.5 mg	13.5 mg	25 mg
Flow rate:	1 mL/min	1 mL/min	0.7 mL/min	0.7 mL/min
Detection:	254 nm	254 nm	254 nm	254 nm
Fraction collection	2 fractions/min	2 fractions/min	2 fractions/min	2 fractions/min
Injection volume fraction analyses	10 µL	5 µL	5 µL	2 µL

The fractions were analyzed under the following conditions:

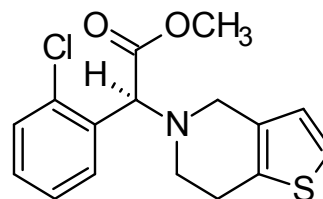
Column : Kromasil AmyCoat 3 µm
(4.6x150 mm)
Mobile phase : Heptane/IPA 90/10
Flow rate : 2 mL/min
Temperature : 20 °C
Detection : 254 nm
Run time : 3 min

3. RESULTS AND DISCUSSION

3.1 Clopidogrel

S-Clopidogrel (Scheme 1) is an oral antiplatelet agent used to inhibit blood clots in coronary artery disease, peripheral vascular disease and cerebrovascular disease [25]. It is sold by Bristol-Myers Squibb and Sanofi under the trade label Plavix. A number of HPLC methods for analysis of clopidogrel and related substances were reported, which include the use of chiral (both protein and small-molecule based) and achiral stationary phase with aqueous organic solvents as eluent. In addition, racemic clopidogrel was separated into its enantiomers by supercritical fluid chromatography [21]. Qualitative RP-HPLC methods were developed for the enantiomeric separation of racemic clopidogrel and related substances using acetonitrile-phosphate buffer mobile phase on CHIRAL-AGP [22] and Chromolith Performance 18e columns. A quantifiable RP-HPLC method was also developed using methanol-phosphate buffer and Sunfire C18 column [24]. In addition, a monograph on clopidogrel API available in the US Pharmacopoeia 29 recommends the use of RP-HPLC method using acetonitrile-phosphate buffer as an eluent on L57 column for the detection of clopidogrel and its impurities. Herein, we report development of scalable

enantioselective normal-phase HPLC method for the separation of racemic clopidogrel. The recovery of the analyte from the organic solvent based mobile phase is much simpler compared to that of the reported acetone/acetonitrile-phosphate buffer mixture. The analytical parameters for the resolution of racemic clopidogrel with n-hexane-ethanol (EtOH) using chiralcel OJ chiral stationary phase already reported in literature [26] but nowhere separation using Amycoat CSP and scale up studies in preparative chromatography documented till now. In the reported process hexane was used as mobile phase which is not a solvent of choice for chromatographic separations. In the current separation heptane was used which is a greener solvent and additionally one can choose any of the techniques (SMB or prep. chromatography) as per suitability and availability of resources, since commissioning and operation of SMB chromatography is quite complicated and costly as well, as compared to prep. Chromatography.



Scheme 1. Structure of Clopidogrel

Herein total, four preparative separations were executed using clopidogrel API on AmyCoat chiral stationary media. In first two separations (Figs. 3 & 5) the mobile phase was used heptane/isopropyl alcohol in 98/2 ratio and the rest separations (Figs. 7 & 9) were carried out using 100% isopropyl alcohol. In the first separation 8.0 mg of compound was loaded, the obtained average purity and recovery 82.6%,

88.2% and 57.9%, 25.0%, in that order for first and second peak (Table 4) while for second separation at 13.5 mg loading the purity and recovery for first and second peaks obtained were 87.4%, 92.2% and 90.9%, 84.4% (Table 5).

The rest of following separations (3 & 4) were carried out using 100% isopropyl alcohol (IPA) in the same chiral stationary phase. As the solubility of API is better in alcohol as compared

to hexane/IPA mixture so more compound was loaded in the same chiral stationary media *i.e.* 13.5 mg and 25 mg (Figs. 7 & 9). In 13.5 mg compound loading, the obtained average purity and recovery of 94.3%, 97.3% and 53.25%, 51.71%, in that order for first and second peak (Table 6) while at 25 mg compound loading the purity and recovery for first and second peaks were 82.7%, 88.1% and 50.0%, 25.0%, respectively (Table 7).

Separation 1

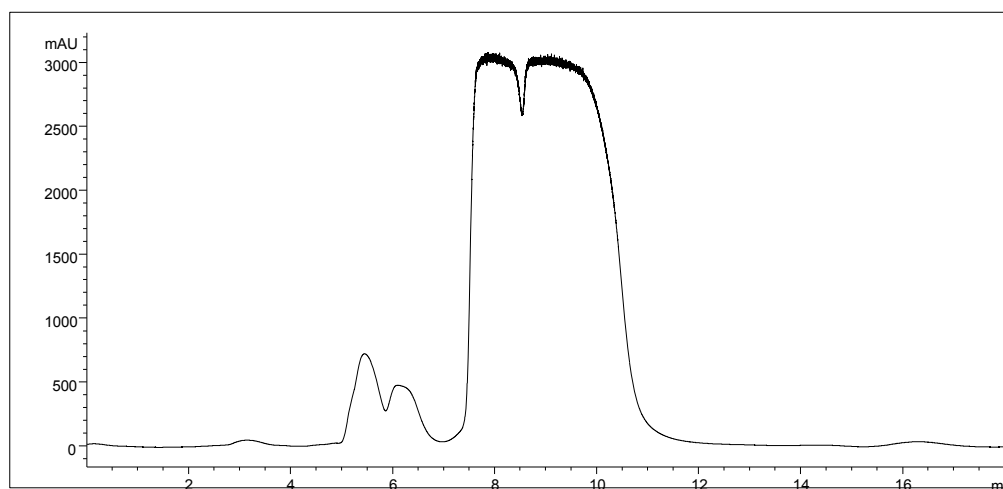


Fig. 3. Preparative chromatogram of Separation 1 (8 mg)

Table 4. Results from fraction analyses of separation 1 with purity / yield calculations for different fraction pools

Fraction	1 st peak	2 nd peak	Sum	Purity 1 st (%)	Purity 2 nd (%)
3	0	0	0		
4	18595.6	1043.6	19639.2	94.7	5.3
5	11991.75	11714.425	23706.18	50.6	49.4
6	6322.425	9818.075	16140.5	39.2	60.8
7	3235.575	7385.25	10620.83	30.5	69.5
8	1556.05	5418.5	6974.55	22.3	77.7
9	576.6	3652.13	4228.73	13.6	86.4
10	178.01	2360	2538.01	7.0	93.0
11	54.25	1351.53	1405.78	3.9	96.1
12	0	610.04	610.04	0.0	100.0
13	0	207.65	207.65	0.0	100.0
14	0	63.4	63.4	0.0	100.0
sum	42510.3	43353.6	65614.6	167.0	

1 st peak			2 nd peak		
Fraction pool	Purity (%)	Recovery (%)	Fraction pool	Purity (%)	Recovery (%)
No 4	94.7	43.7	No 9-12	91.1	18.4
No 4-5	70.6	72.0	No 8-12	85.2	31.5

Prep Separation of Clopidogrel on AmyCoat 10 µm (8 mg)

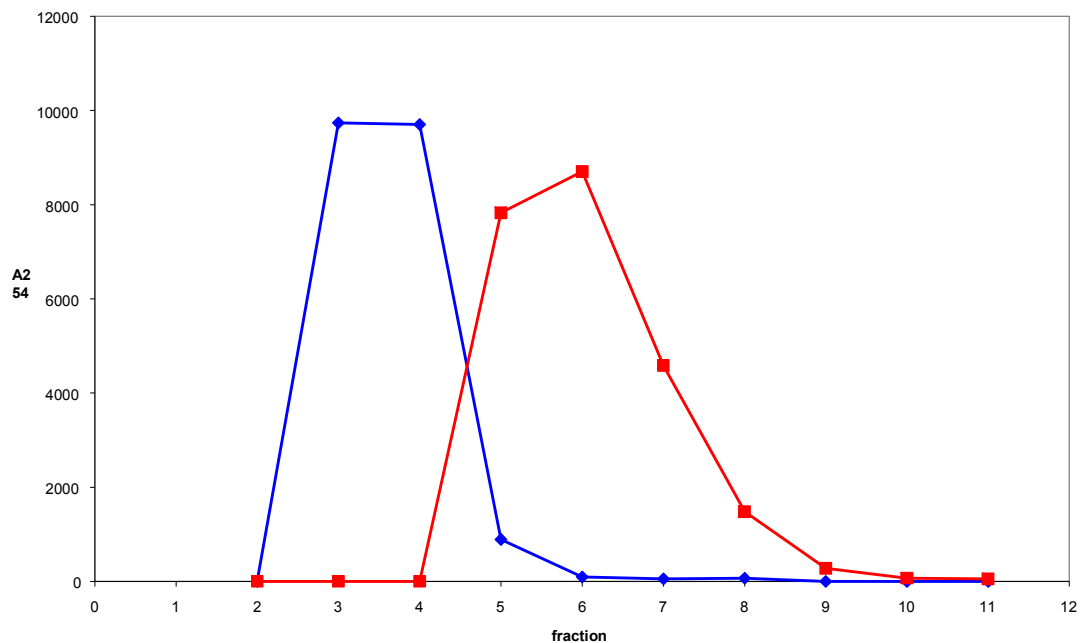


Fig. 4. Reconstructed elution profile of separation 1

Separation 2

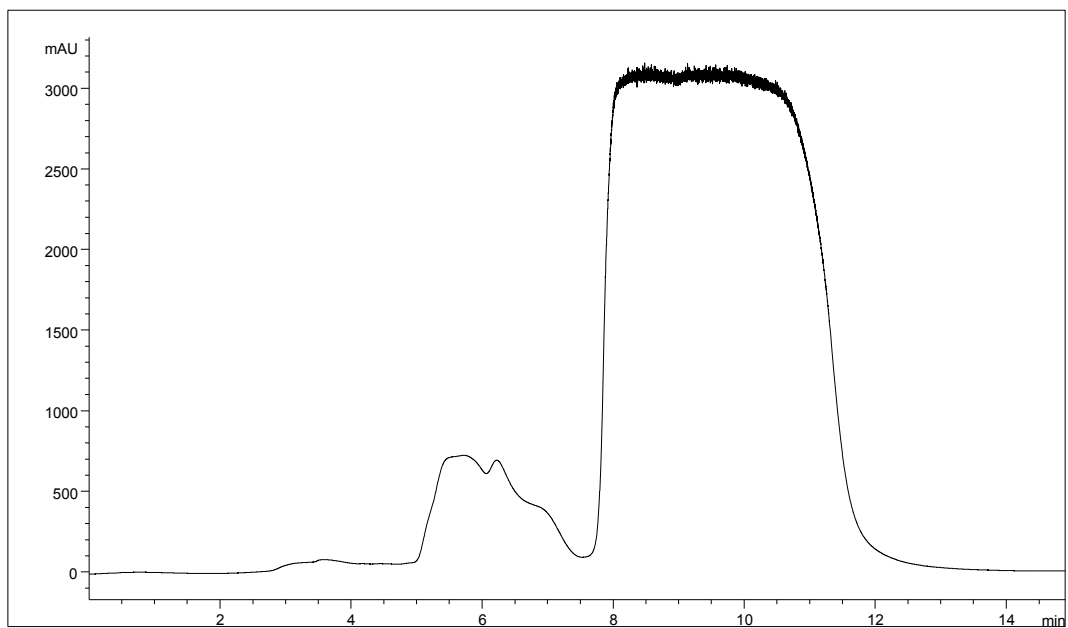


Fig. 5. Preparative chromatogram of Separation 2 (13.5 mg)

The presented results show that the racemate Clopidogrel can be separated by means of Kromasil Amy Coat. The heptane/IPA 98/2 (v/v) mobile phase rendered higher selectivity and

therewith also better purity/yield results under batch chromatography mode. The anticipated productivity is in the range of 26 g racemate per kg of stationary phase and hour. The other tested

mobile phase consisting of 100% IPA rendered somewhat lower selectivity is however a significantly better solvent for the Clopidogrel racemate. With pure IPA, the solubility was found to be 50 mg/mL, whereas only about 15 mg of the racemate could be dissolved in heptane/IPA 98/2 (v/v). Thus, IPA is the preferred mobile

phase for SMB-type separation, as lower selectivity is required (compared to batch chromatography) in order to still be able to isolate pure enantiomers as extract and raffinate as indicated by reconstructed elution profiles (Figs. 4, 6, 8 & 10).

Table 5. Results from fraction analyses of separation 2 with purity / yield calculations for different fraction pools

Fraction	1 st peak	2 nd peak	Sum	Purity 1 st (%)	Purity 2 nd (%)
3	0	0	0		
4	249.59	0	249.59		
5	6867.75	0	6867.75	100.0	0.0
6	8518.08	74.73	8592.81	99.1	0.9
7	2955.48	6010.71	8966.19	33.0	67.0
8	239.4	6743.85	6983.25	3.4	96.6
9	0	4472.53	4472.53	0.0	100.0
10	0	1877.9	1877.9	0.0	100.0
11	0	495.32	495.32	0.0	100.0
12	0	83.07	83.07	0.0	100.0
sum	18830.3	19758.1	38255.8	235.5	

1st peak

Fraction pool	Purity (%)	Recovery (%)
No 4-6	99.5	83.0
No 4-7	75.3	98.7

2nd peak

Fraction pool	Purity (%)	Recovery (%)
No 7-12	86.0	99.6
No 8-12	98.3	69.2

Prep Separation of Clopidogrel on AmyCoat 10 μ m (13.5 mg)

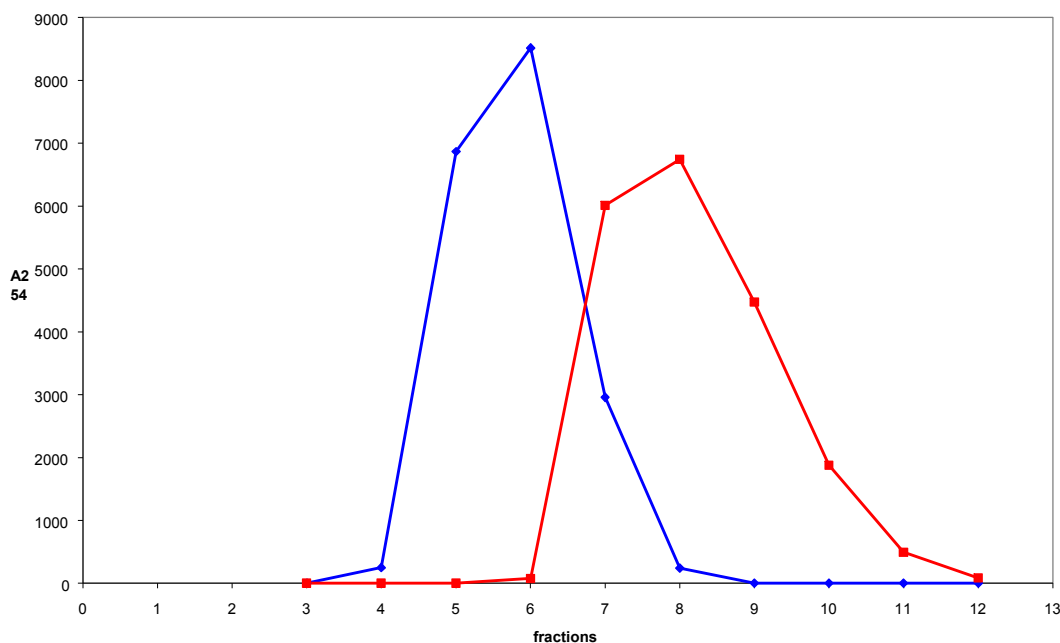


Fig. 6. Reconstructed elution profile of separation 2

Separation 3

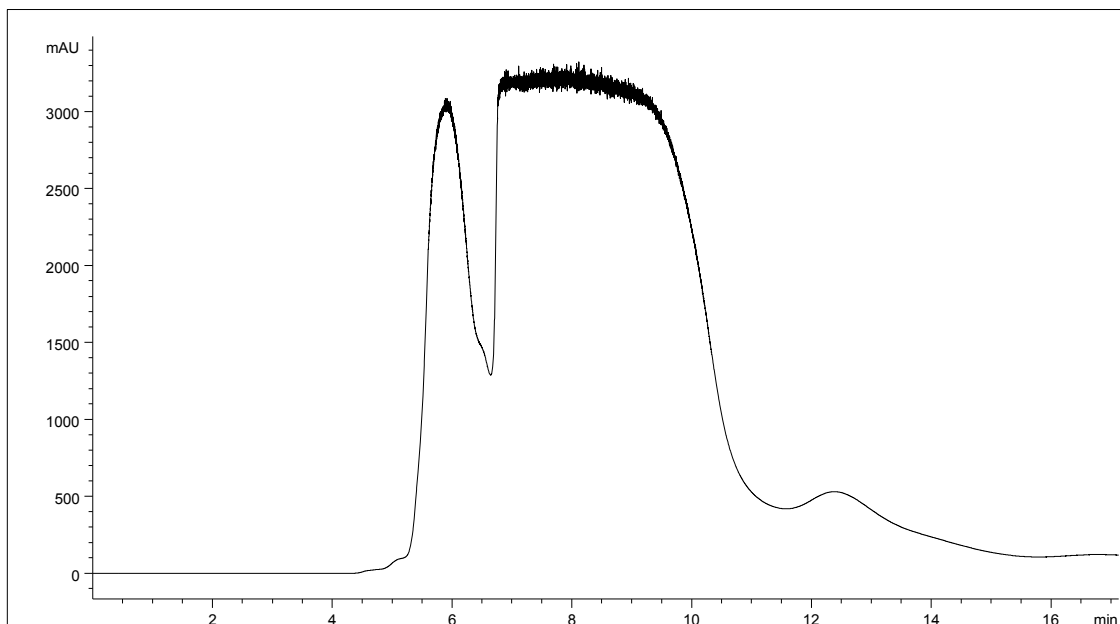


Fig. 7. Preparative chromatogram from separation 3 (13.5 mg)

Prep Separation of Clopidogrel on AmyCoat 10 μ m (13.5 mg in IPA)

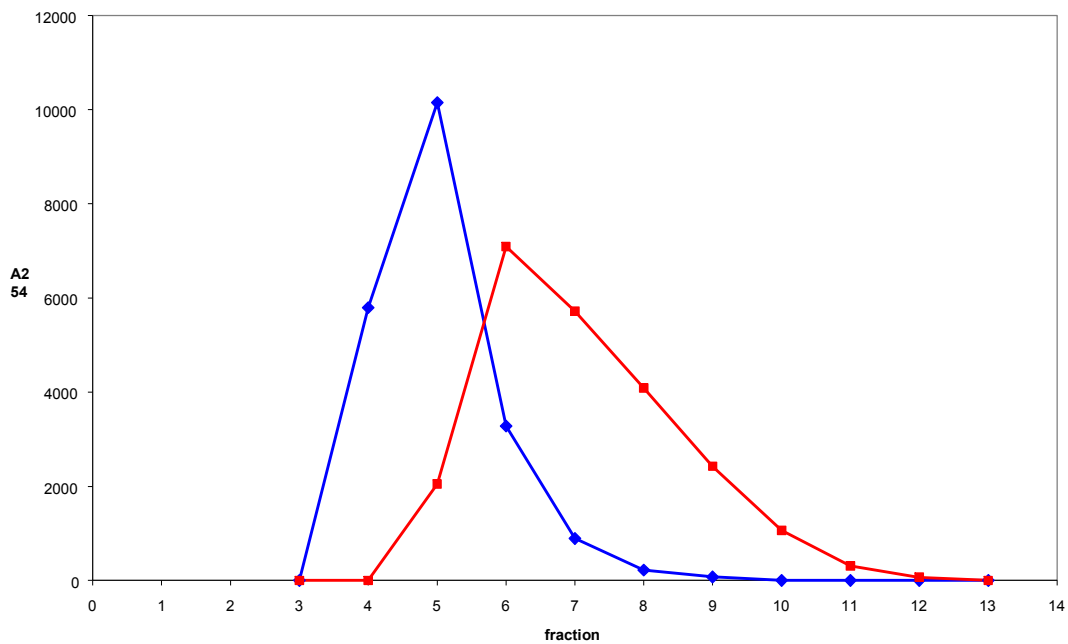


Fig. 8. Reconstructed elution profile of Separation 3 (13.5mg)

Separation 4

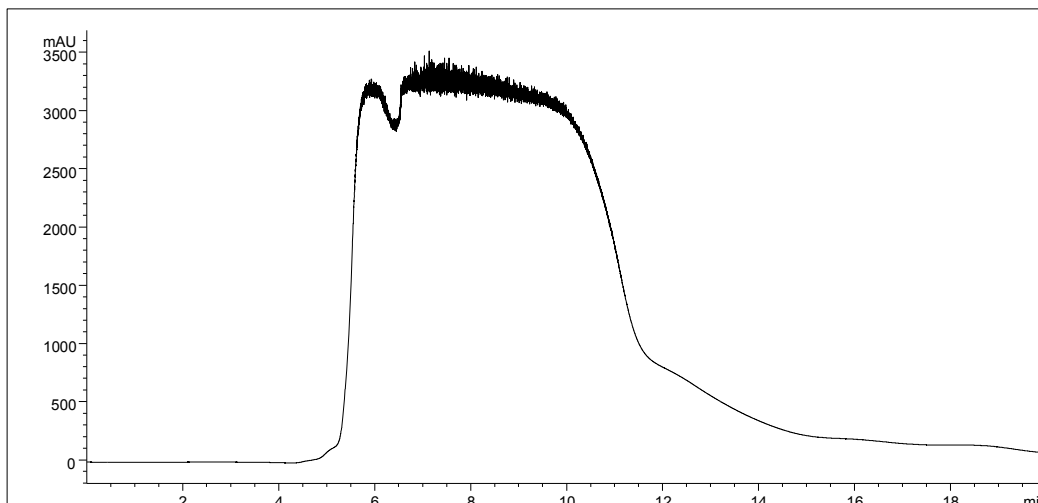


Fig. 9. Preparative chromatogram from separation 4

Prep Separation of Clopidogrelon AmyCoat 10 µm (25 mg in IPA)

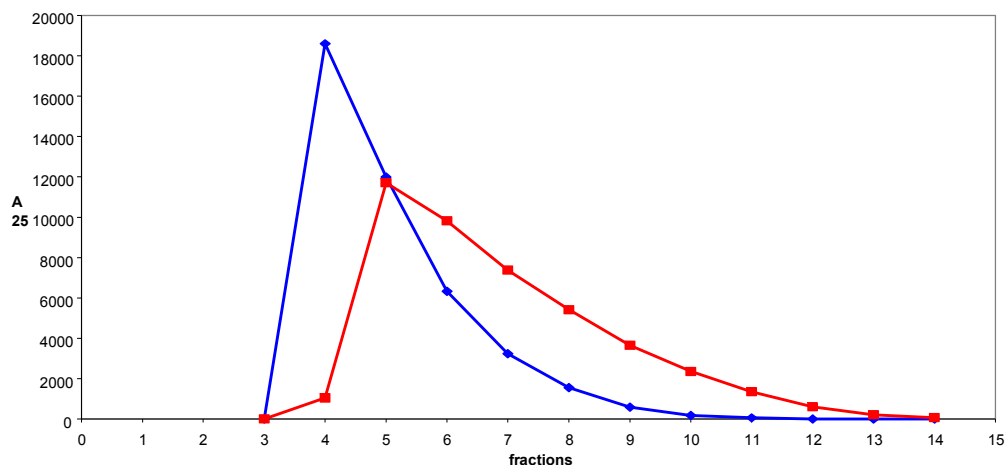


Fig. 10. Reconstructed elution profile of separation 4

Table 6. Results from fraction analyses of separation 3 with purity / yield calculations for different fraction pools

Fraction	1st peak	2nd peak	Sum	Purity 1st (%)	Purity 2nd (%)
3	0	0	0		
4	5791.32	0	5791.32		
5	10149.3	2047	12196.3	83.2	16.8
6	3280.23	7089.7	10369.93	31.6	68.4
7	892.03	5714.36	6606.39	13.5	86.5
8	218.77	4084.99	4303.76	5.1	94.9
9	73.34	2421.12	2494.46	2.9	97.1
10	0	1060.27	1060.27	0.0	100.0
11	0	306.997	306.997	0.0	100.0
12	0	67.66	67.66	0.0	100.0
13	0	0	0		
sum	20405.0	22792.1	37338.1	136.4	

1 st peak			2 nd peak		
Fraction pool	Purity (%)	Recovery (%)	Fraction pool	Purity (%)	Recovery (%)
No 4	100.0	28.4	No 9-12	98.1	16.9
No 4-5	88.6	78.1	No 8-12	96.5	34.8

Table 7. Results from fraction analyses of separation 4 with purity / yield calculations for different fraction pools

fraction	1 st peak	2 nd peak	sum	purity 1 st (%)	purity 2 nd (%)
3	0	0	0		
4	18595.6	1043.6	19639.2	94.7	5.3
5	11991.75	11714.425	23706.18	50.6	49.4
6	6322.425	9818.075	16140.5	39.2	60.8
7	3235.575	7385.25	10620.83	30.5	69.5
8	1556.05	5418.5	6974.55	22.3	77.7
9	576.6	3652.13	4228.73	13.6	86.4
10	178.01	2360	2538.01	7.0	93.0
11	54.25	1351.53	1405.78	3.9	96.1
12	0	610.04	610.04	0.0	100.0
13	0	207.65	207.65	0.0	100.0
14	0	63.4	63.4	0.0	100.0
sum	42510.3	43353.6	65614.6	167.0	

1 st peak			2 nd peak		
Fraction pool	Purity (%)	Recover (%)	Fraction pool	Purity (%)	Recovery (%)
No 4	94.7	43.7	No 9-12	91.1	18.4
No 4-5	70.6	72.0	No 8-12	85.2	31.5

4. CONCLUSION

HPLC method has been developed for chiral separation of Clopidogrel using Amycoat chiral stationary phase. Heptane/IPA (98/2, v/v) eluent in Amycoat CSP is suitable for separation using batch chromatography, since the capacity factor and selectivity is higher as compared to, in 100% IPA eluent using the same CSP, the later can be a better choice for separation of racemic API in SMB, as is a high performance separating device can even separate the peaks having poor chromatographic selectivity. Recycling will also be easier and economical using a single solvent *i.e.* 100% IPA in SMB. Therefore the racemic API can be resolved into its enantiomers and scaled up using the same chiral stationary media by means of any of the techniques as per availability and suitability of resources.

COMPETING INTEREST

Authors have declared that no competing interests exist.

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