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Chiral Analysis Control of Three Nonsteroidal Anti-inflammatory Drugs by HPLC Methods

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ABSTRACT

The liquid chromatographic enantiomer separation of three (unused, used and expired) nonsteroidal anti-inflammatory drugs (NSAIDs) was performed on covalently immobilized and chiral stationary phases (CSPs) (Chiralpak IA and Chiralpak IB) and coated-type CSPs (Chiralpak AD, Chiralcel OJ, Chiralcel OD-H and Chiralcel OD) derived from polysaccharide derivatives. The results indicated that Chiralpak AD showed higher enantiomer separation than other column.

Keywords: HPLC, Anti-inflammatory drugs, Nonsteroidal

INTRODUCTION

Nonsteroidal Anti-Inflammatory Drugs (NSAIDS) constitute a wide group of pharmaceutical products of different chemical classes including salycilates, pyrazol, oxicams, fenamates, arylacetic acids and arylpropionic acids. NSAIDS are used meinly for the treatment of pain and inflammation because they have anti-pyretic, anti-inflammatory and analgesic effects [1-5].

All compoundsin this class acts by inhibiting COX enzymes, which are involved in inflammation and are responsible for the synthesis of prostaglandins [6,7].

Many studies have demonstrated the potential applications of High Performance Liquid Chromatography (HPLC) for the separation of pharmaceutical samples. Sinceit shows a method for purification and quantification of enantiomers. Indeed, it allows the separation of chiral compounds on chiral stationary phases or chiral reagents after addition of the mobile phase [8-10].

Many chiral selectors have been discovered and utilized successfully. More PSC 200 is commercially available with the chiral selector coated, or immobilized on a grafted silica gel. Resolution of the enantiomers of these phases is a function of the difference in stability of the complexes formed between diastereomeric enantiomers and the chiral selector [11].

Polysaccharide (cellulose and amylose) derived Chiral Stationary Phases (CSPs) have proved their usefulness as chiral selector in High Performance Liquid Chromatography (HPLC), and a wide range of enantiomer compounds have been separated on these CSPs [12,13]. Over the past few years, it has been demonstrated that the Chiral Stationary Phases (CSPs) separating many of compounds in our laboratory [14-20]. In this study, we present the comparative of three nonsteroidal anti-inflammatory drugs in different stage unused, used and expired drug [12,13].

EXPERIMENTAL

The tablet was diluted in ethanol followed by a simple filtration.

Instruments and reagents

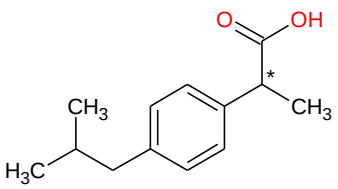
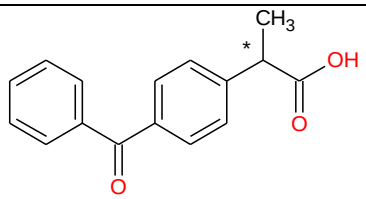
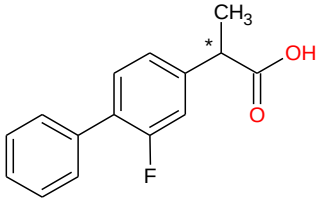
Chromatography was performed at room temperature using an HPLC Shimadzu 20A.
HPLC analysis was carried out using a chromatographic system composed of a LC-20AD pump, aSPD-20ADdetectorand aRheodyne model 1907 injector with 20 µl loop. Ethanol, hexane and isopropanol (HPLC-Grade) were obtained from Sigma-Aldrich.

Ibuprofenwas purchased from (BIOGARAN laboratory), Flurbiprofen (GEO-Pharm laboratory), Ketoprofen (Neomedic laboratory).

Chromatographic conditions

Chiralcel® OD, Chiralcel® OD-H, Chiralpak® IA, Chiralpak® IB, Chiralpak® AD, Chiralcel® OJ, column (250 × 4.6 mm) were purchased from eChiral Technologies Europe (Illkirch Cedex, France). The chromatographic conditions were as follows: at a flow rate of 0.4 ml min^{-1} and detection at the wavelength of 254 nm, 2-propanol/hexane and hexane were used as a standard mobile phase on all CSPs (Table 1).

Table 1: The molecular structure and commercial name of the PPs

Compound	Molecular structure	Commercial name
Ibuprofen(RS)-2-(4-(2-methylpropyl)phenyl) propanoic acid		BIOGARAN AMADVIL®
Ketoprofen(RS)-2-(3-benzoylphenyl)propanoic acid		KETOFEN® PROFEN®
Flurbiprofen (RS (2- (3-fluoro-4-phenylphenyl)		FLUBIFEN ® ANTADINE®

RESULTS AND DISCUSSION

The goal of this study was to investigate the separation of NSAIs using high performance liquid chromatography.
We made a separation of the three anti-inflammatory active ingredients with six chiral columns based polysaccharides by varying the mobile phase. The results obtained after HPLC analyses are shown in the table which follows.

Rs resolution factor; tr1 retention time of the first enantiomer eluted; tr2, retention time of the second enantiomer eluted; α separation factor A1 surface of the first peak.

The results of Table 2 show that the separation of ibuprofen is done with column Chiralpak® AD, IA and IB and Chiralcel® OD and OJ, but any separation with the Chiralcel® OD-H.

The best separation of the active ingredient is done with the stationary phase Chiralcel® OD unfortunately this has been removed because it contains impurities, for this we will do the separation on the column Chiralpak® AD.

Comparing the results obtained with columns Chiralcel® OD and Chiralcel® OD-H, which are made of the same chiral selector but these differ in the diameter of the silica gel particles, there is no separation of the three active principles on both columns except for ibuprofen on Chiralcel® OD.

The retention time of the three products on the column Chiralpak® OD-H is slower relative to other columns because we worked with hexane as mobile phase and a flow rate of 0.3 ml/min.

The flurbiprofen was separated on the columns Chiralpak® AD, IA and IB and Chiralcel® OD and OJ. The Chiralpak® IA provides good separation (α=1.44) with high resolution (Rs=3.12).

The active principle ketoprofenwas separated on the columns Chiralpak® IA, IB, AD but any separation on the Chiralcel® OD-H, OD, OJ. The high resolution of this active principle was Rs=1.77 on the Chiralpak® AD.

Following the results is summarized in the best factors obtained by the six columns: flurbiprofen on Chiralpak® IA (Rs=3.12, α=1.44), ibuprofen on Chiralpak® AD (Rs=1.73, α=1.08), ketoprofen on Chiralpak® IA (Rs=1.77, α=1.08).

The results obtained by the column Chiralpak® AD for all active principle (tr1, tr2, K, R, α) are almost close: ibuprofen, ketoprofen and flurbiprofen respectively (tr1=38.82, 39.24, 39.33); (tr 2=41.41, 41.85, 41.99); (Rs=1.73, 1.77, 1.76); K '=3.75, 3.27, 3.31); (α=1.08, 1.08, 1.08).

Table 2: Chromatographic results of unused anti-inflammatory drugs

Columns		Ibuprofen	Ketoprofen	Flurbiprofen
Chiralcel® OD	**t_1**	8.63	11.55	9.81
	t_2	9.76	-	10.52
	K'$_1$	0.06	0.18	-
	K'$_2$	0.20	-	0.07
	α	0.99	-	-
	Rs	2.17	-	0.96
	A_1	44.14	99.62	87.72
	A2	45.05	-	12.27
Mobile phase		Hexane		
Chiralcel® OD-H	**tr_1**	52.02	51.77	90.51
	tr_2	-	-	-
	K'$_1$	0.23	-	0.28
	K'$_2$	-	-	-
	α	-	-	-
	Rs	-	-	-
	A_1	88.61	100	99.51
	A2	-	-	-
Mobile phase		Hexane		
Chiralcel® OJ	**tr_1**	8.630	10.404	9.784
	tr_2	10.431	-	10.477
	K'$_1$	0.059	0.204	0.200
	K'$_2$	0.280	-	0.285
	α	1.428	-	1.425
	Rs	1.341	-	1.200
	A_1	44.981	99.875	32.860
	A2	54.783	-	67.098
Mobile phase		Hexane		
Chiralpak® IB	**tr_1**	21.24	6.74	9.91
	tr_2	22.39	8.51	10.32
	K'$_1$	0.51	-	0.38
	K'$_2$	0.60	0.26	0.43
	α	1.15	-	1.14
	Rs	1.55	0.45	1.34
	A_1	41.28	36.02	8.34
	A2	43.60	37.68	8.63
Mobile phase		Hexane	Hexane	Hexane/2-propanol
Chiralpak ® IA	**tr_1**	10.05	14.74	12.76
	tr_2	10.37	15.03	15.00
	K'$_1$	0.34	0.94	0.65
	K'$_2$	0.38	0.98	0.95
	α	1.12	1.04	1.44
	Rs	1.11	0.33	3.12
	A_1	43.99	44.86	43.79
	A2	46.00	48.83	47.88
Mobile phase		Hexane/2-propanol 50/50		
Chiralpak® AD	**tr_1**	38.82	39.24	39.33
	tr_2	41.41	41.85	41.99
	K'$_1$	3.45	3.00	3.04
	K'$_2$	3.75	3.27	3.31
	α	1.08	1.08	1.08
	Rs	1.73	1.77	1.76
	A_1	32.98	48.32	41.24
	A2	32.45	48.84	40.06
Mobile phase		hexane		

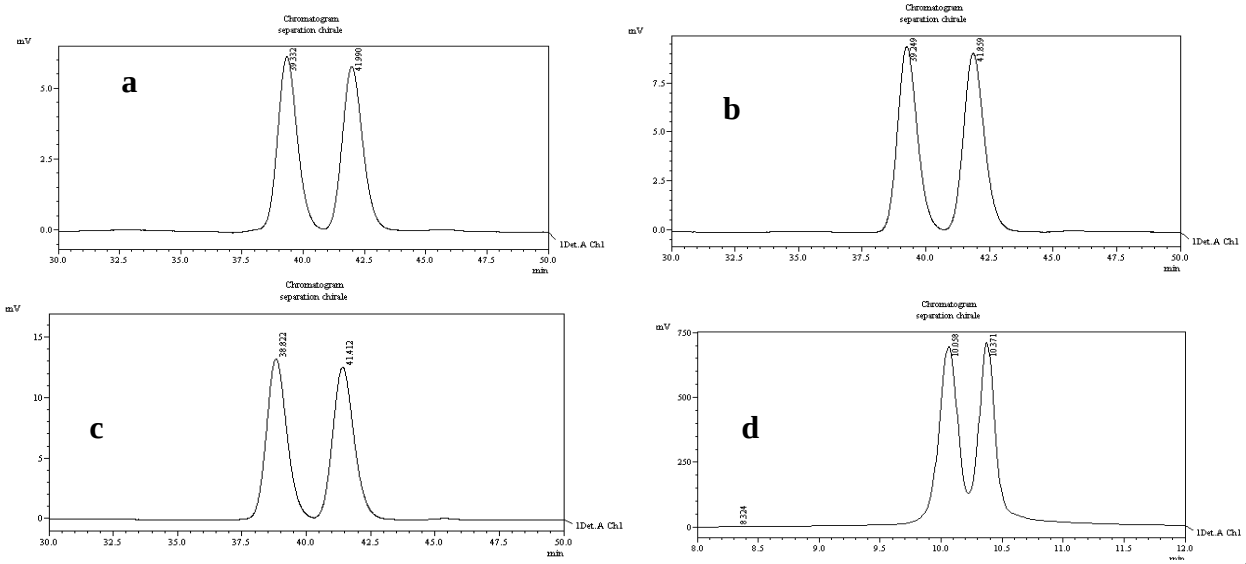


Figure 1: Chromatograms of enantiomer separation of a) flurbiprofen, b) Ketoprofen, c) Ibuprofenunused on Chiralpak® AD, d) Ibuprofen unused on Chiralpak® IA

Mobile phase: hexane; flow rate: 0.4 ml min^{-1}; detection wavelength: 254 nm; injection amount: 20 µl.

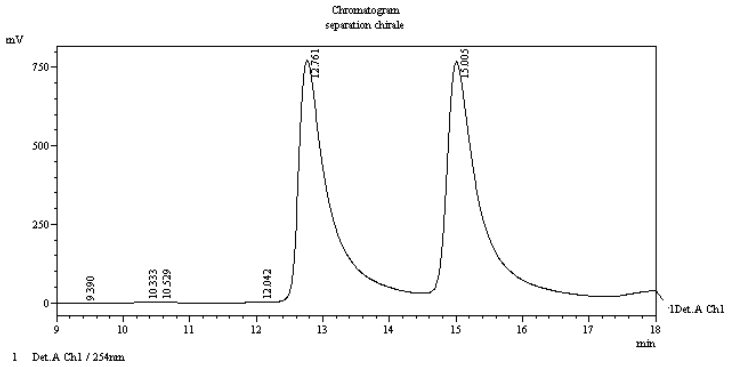


Figure 2: Chromatograms of enantiomer separation of Flurbiprofen unused on Chiralpak® IA

Mobile phase: hexane/2-propanol (50/50); flow rate: 0.4 ml min^{-1}; detection wavelength: 254 nm; injection amount: 20 µl.

Table 3: Chromatographic result of the drugs used on the column Chiralpak® AD

Columns		Ibuprofen	Ketoprofen	Flurbiprofen
		Biogaran	Profen	Antadine
Chiralpak® AD	tr1	27.15	39.13	18.54
	tr2	33.78	41.76	19.78
	K'_1	2.11	4.16	0.65
	K'_2	2.87	3.26	1.03
	α	1.35	1.08	1.14
	Rs	1.46	1.77	1.95
	A1	47.27	38.29	46.58
	A2	47.47	34.01	46.98
Mobile phase		Isopropanol/Hexane 50/50	Hexane	Isopropanol/Hexane 20/80

Table 4: Chromatographic results of expired drugs on the column Chiralpak® AD

Columns		Ibuprofen		ketoprofen
		Amadvil	Apifen	Profen
Chiralpak® AD	tr1	38.17	21.18	30.84
	tr2	40.48	23.16	36.22
	K'_1	2.78	0.22	2.87
	K'_2	3.64	1.65	2.70
	α	1.07	1.50	1.25
	Rs	1.61	2.23	1.53
	A1	38.35	39.09	47.37
	A2	39.00	59.69	47.36

Mobile phase	Hexane	Hexane/Isopropanol	Hexane

The results presented in Tables 3 and 4 indicate that Chiralpak® AD column gives a good resolution for the drugs used (Biogaran, profen, antadine) (R=1.95 for flurbiprofen). The active ingredients are racemic mixtures because the percentage enantiomeric not from the exchange surfaces of each compound for example ibuprofen (A1=47.27, A2=47.47).

The last table shows the expired drug separation values (Profen, amadvil and apifen) on the stationary phaseChiralpak® AD, these results show that amadvil, apifen and Profendrug are racemic mixtures and shows no change after the expiry in enantiomerically percentage.

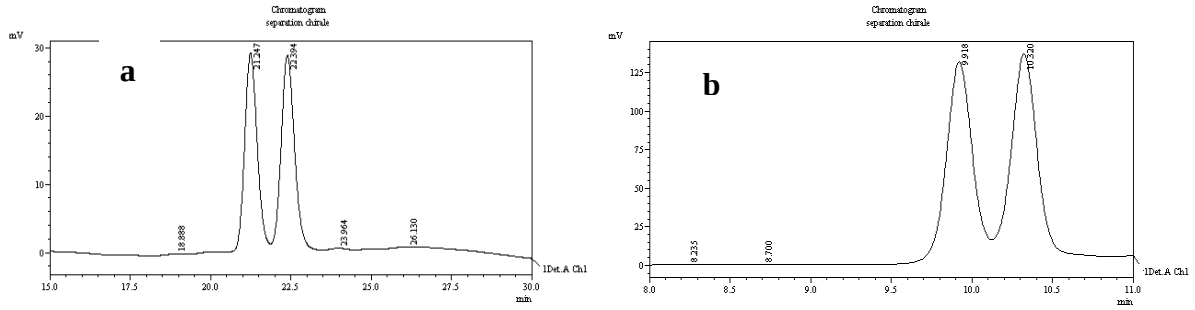
A mechanism of chiral separation

Polysaccharides such as cellulose and amylose have a high potential for application in chiral separation. Indeed, these polysaccharides have an asymmetrical spiral structure composed of n units of D-(+)-glucose linked by β bridges (1-4) for the cellulose and bridges by α (1-4) to the amylose. Each of these units contributes to the chiral recognition of solutes with which it interacts [21].

Resolution of the enantiomers of these phases depends on the difference in stability of the complexes formed between the diastereomeric enantiomers and the chiral selector. The chiral recognition process is based on interactions between different enantiomer and the stationary phase, such as hydrogen bonds, π-π interactions, dipole-dipole interactions, hydrophobic effect, ionic interactions and steric effects.

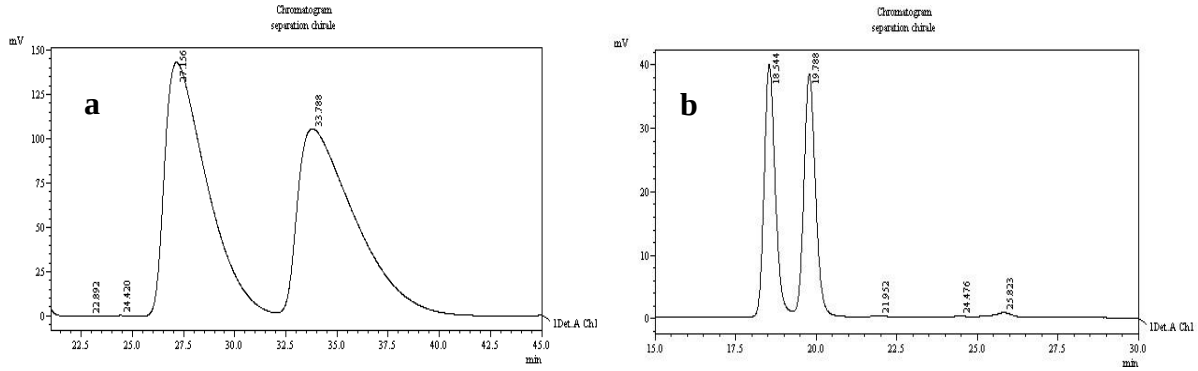
This chiral recognition was explained in 1933 by Easson and Stedman who introduced the notion of interaction in three points to explain the differences in activity related to the stereochemistry. This notion was echoed later by Ogston and Dalgliesh [22]. This concept of chiral recognition indicates that the two enantiomers have different affinities for the same receiver. This affinity is based on a minimum of three sites of interaction between the solute and the receiver. The receiver will be able to differentiate between the two enantiomers as a single enantiomer will have a satisfactory complementarity [23].

Our products are acids therefore they exist π-π interactions, hydrogen bonds and dipole-dipole interactions between the PSCs, and the three active principles. Thus the π-π interaction may play an important role in the enantioseparation of profens on cellulose tris (3, dimethylphenylcarbamate). The presence of the alcohol in the mobile phase is a competition with the chiral stationary phase for the formation of hydrogen bonds with the analytes.



Mobile phase: hexane; flow rate: 0.4 ml min^{-1}; detection wavelength: 254 nm; injection amount: 20 µl.

Figure 3: Chromatograms of enantiomer separation of a) Ibuprofen, b) flurbiprofen unused on Chiralpak® IB



Mobile phase: a) 1): hexane,2) 2-propanol/hexane v/v: 50/50, flow rate: 0.4 ml min^{-1}; b) hexane, flow rate: 0.3 ml min^{-1}; detection wavelength: 254 nm; injection amount: 20 µl (Figures 1-5).

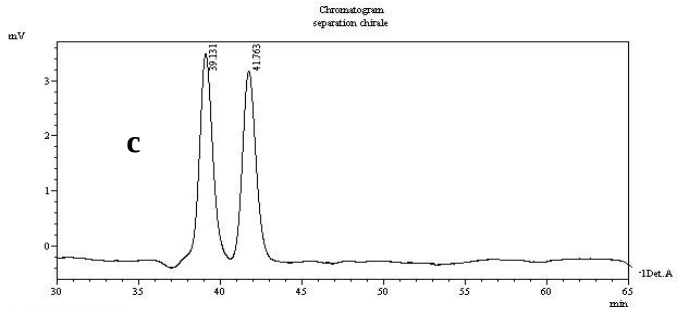


Figure 4: Chromatograms of enantiomer separation of a) Ibuprofen, b) flurbiprofen, c) ketofenused on Chiralpak® AD
Mobile phase: a) and c): hexane, flow rate: 0.4 ml min^{-1}; b) 2-propanol/hexane v/v: 20/80, flow rate: 0.3 ml min^{-1}; detection wavelength: 254 nm; injection amount: 20 µl

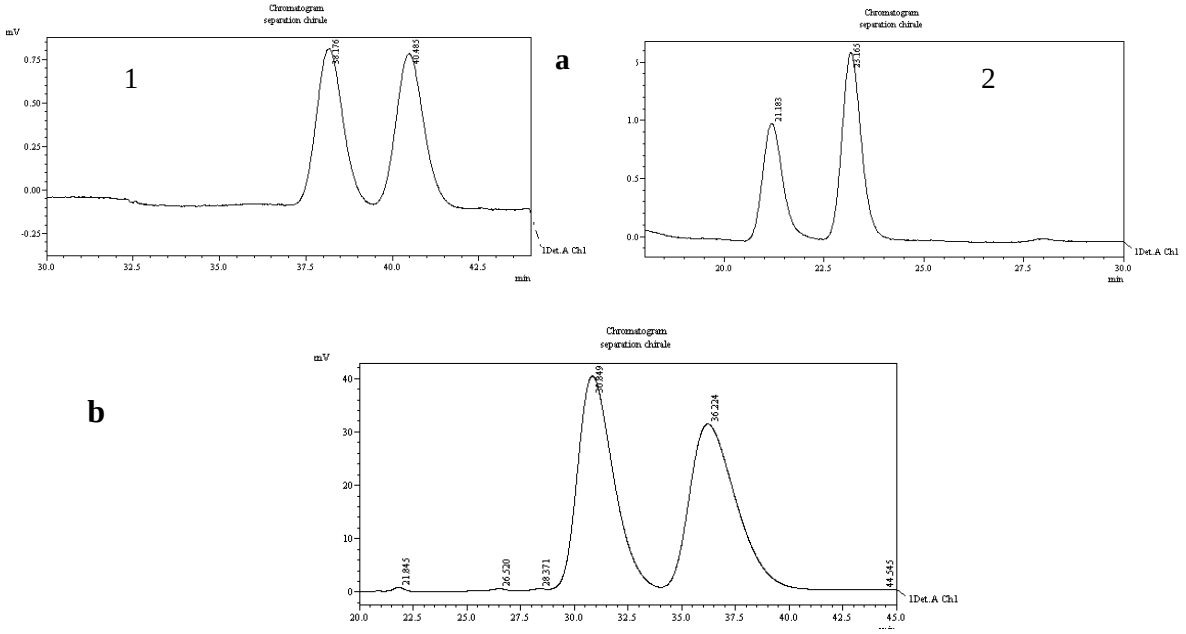


Figure 5: Chromatograms of enantiomer separation of a) Ibuprofen 1) Amadvil, 2) Apifen, b) ketofen (Profen) expired on Chiralpak® AD

CONCLUSION

The results of this study confirm that the liquid chromatography can separate pharmaceuticals product using chiral stationary phases derived from polysaccharides. In addition they indicated that there's no change in the racemic mixture of each compound is used or expired;so the drugs remain in the racemic form. Finally the column Chiralpak® AD gives a good separation of the three non-steroidal anti-inflammatory drugs.

REFERENCES

[1] G. Carlucci, A. Antonio D'Archivio, M. Anna Maggi, P. Mazzeo, F. Ruggieri, *Analytica. Chemica. acta*., **2007**, 601, 68-76.
[2] H. Shaaban, T. Górecki, *Analytica Chemica Acta.*, **2011**, 702: 136-143.
[3] M. Dawod, C. Michael, *J. Chromatography A.*, **2008**, 278-284.
[4] Chao-Hsiang Hsu, Yi-Jie Cheng, Brenda Singco, Hsi-Ya Huang, *J. Chromatography A*, 1218, **2011**, 350-358.
[5] R. Dills **2012**, *Pharmaceut. Res.*, 65, 5-8.
[6] E. Sandilands, **2015**, *Elsevier,* 44, 3, 185-186.
[7] K. MacCormack, *Pain*, **1994**, 59, 9-43.
[8] I. Tomoyuki I. Ed. Springer Science & Business Media, **2010**, 1-352.
[9] T. Arai, *Chromatogra. B.*, **1998**, 717, 295-311
[10] M.M. Hefnawy, *Chromatogra. B.*, **2007**, 856, 328-336.
[11] P. Morin *Annales Pharmaceutiques Françaises.*, **2009**, 67: 241-250.
[12] C. Yamamoto, *Pure and Applied Chemistry*, **2007**, 79, 1561-1573.
[13] C. De-Miao, *Chin. J. Anal. Chem*., **2007**, 35: 75-78.
[14] N. Belboukhari, A. Cheriti, C. Roussel, N. Vanthuyne, *Nat. Prod. Res.*, **2010**, 247, 669-681.

[15] I. Rahou, N. Belboukhari, K. Sekkoum, A. Cheriti, H.Y. Aboul Enein, *Chromatographia*., **2014**, 77, 17-18.
[16] N. Belboukhari, I. Rahou, A. Cheriti, A. Benmiloud, N. Cheikh, O. Fandougouma, *Phyto. Chem. Biol. Sub.*, **2011**, 5, 170-173.
[17] N. Lahmar, N. Belboukhari, A. Cheriti, *Int. J. Chem. Stud*., **2015**, 1, 1-5.
[18] N. Belboukhari, *J. Chromat. Sep. Techniq.*, **2011**, 3, 67-68.
[19] K. Addadi, K. Sekkoum, N. Belboukhari, A. Cheriti, H.Y. Aboul-Enein, **2015**, *Chirality*, 27, 332-338.
[20] Ismahane Rahou, Khaled Sekkoum, Nasser Belboukhari, AbdelkrimCheritI, Hassan Y. Aboul-Enein *chromatographic sci.*, **2016**, 1-7.
[21] C. Yamamoto, *Pure Appl. Chem*., **2007**, 79, 1561-1573.
[22] L. Zhou, *J. Pharmaceut. Biomed. Anal*., **2009**, 49, 964-969.
[23] J. Keogh**,** *Pharmacol*., **2010**, McGraw Hill, 1-384.