Enantioselective HPLC separation of bioactive C5-chiral 2-pyrazolines on lux amylose-2 and lux cellulose-2: Comparative and mechanistic approaches

Mohammed Farrag El-Behairy and Aida A. El-Azzouny
Pharmaceutical and Drug Industries Research Division, Medicinal and Pharmaceutical Chemistry Department, National Research Centre, Giza, Egypt

ABSTRACT
Stereoselective analytical HPLC separations have been developed for a series of biologically active chiral 2-pyrazolines (1-22) to be used in monitoring their resolution reactions or to custom semipreparative HPLC separations prior to biological assessment of both enantiomers. Polysaccharide-based chiral stationary phases (CSPs), namely, Lux amylose-2 and cellulose-2, have been used. Both normal (n-hexane/ethanol) and polar organic (ethanol, methanol, acetonitrile, or mixtures thereof) elution modes were very beneficial for the achievement of baseline separations. The impact of various chemical moieties embedded in the structures of 2-pyrazolines 1-22 and the adopted stationary phases on chiral recognition has been investigated. A case of reversed order of elution following alterations in either stationary phase or elution mode has been observed. Our findings recommend that normal elution mode can be used for optimizing semipreparative HPLC methods whereas polar organic mobile phases (such as acetonitrile and ethanol) are more suited to stereoselective reactions monitoring, routine quality control work, or for pharmacological and toxicological assays. These results settle the implementation of polysaccharide-based CSPs using different elution modes and declare the practicality of such CSPs in stereoselective HPLC.

KEYWORDS
Chiral HPLC; elution order; enantioselective; lux column; 2-pyrazolines

CONTACT
Mohammed Farrag El-Behairy mohabeha@gmail.com Pharmaceutical and Drug Industries Research Division, Medicinal and Pharmaceutical Chemistry Department, National Research Centre, ID 60014618, 12622 Dokki, Giza, Egypt.
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**Introduction**

Enantioselective HPLC on chiral stationary phases (CSPs) has become a powerful tool in the drug discovery process, where it is used not only for chiral analyses but also for the fast attainment of enantiopure drug candidates for preliminary comparative biological testing of bioactive enantiomers.\(^1\)\(^-\)\(^3\) The impetus for reliance on chromatography has been fueled by advances in CSPs that allowed for the reliable, robust, and efficient resolution of milligram to gram scale of chiral drug molecules in a timely fashion.\(^4\)

The prochiral pharmacophoric moiety 2-pyrazolines (4,5-dihydro-1H-pyrazole) is a crucial part of numerous drug structures with a wide variety of pharmacological activities such as anticancer,\(^5\) analgesic,\(^6\) hypoglycemic,\(^7\) antitumor,\(^8\) anticonvulsant,\(^9\) anti-inflammatory,\(^10\) antibacterial\(^11\) and antifungal activities.\(^12\) It has been well established that the absolute configuration of the chiral center at C-5 of the 5-substituted 2-pyrazolines can be an important modulator of their biological activities.\(^13\) In previous studies, we have reported on the synthesis of libraries of N-substituted 5-(1,3-benzodioxol-5-yl)-3-tert-butyl-4,5-dihydro-1H-pyrazole derivatives (Figure 1) that showed potent activity in the racemic form.\(^9\),\(^11\) The pharmacological and toxicological properties of pure enantiomers of these derivatives are therefore very interesting to be assessed. Pure enantiomers could be achieved through either asymmetric synthesis or stereoselective resolution of racemates. In both cases, chiral analytical assays are requisite either to evaluate the stereochemical purity of the stereoselective synthesis products or to carry out enantioselective resolution of racemates. Also, chiral bioanalytical assays may be required for further *in vitro* and *in vivo* investigations.

In continuation of our previous studies on 2-pyrazoline candidates 1-22, we discuss here the development of chiral analytical techniques of compounds (1-22) under a variety of conditions. The potential of two polysaccharide-based chiral columns (Lux amylose-2 and cellulose-2) has been investigated. Also, different mobile-phase compositions were used to explore the effect of solvents on the stationary phase's selectivity and recognition abilities.

**Experimental**

**Materials and reagents**

Compounds (1-22) were synthesized according to the reported procedures.\(^9,\)\(^11\) HPLC grade *n*-hexane, methanol, ethanol, acetonitrile, and 2-propanol were from Merck (Darmstadt, Germany) and Sigma chemicals (St. Louis, MO, USA).

**Instrumentation and analytical conditions**

The HPLC unit was Agilent 1100 series apparatus equipped with a quaternary pump, a vacuum degasser, autosampler,
column compartment, and a diode array UV-detector. The signal was acquired and processed by HP Chemstation software. The columns used were Lux amylase-2 (amylose tris (5-chloro-2-methylphenylcarbamate)) and Lux cellulose-2 (cellulose tris(3-chloro-4-methylphenylcarbamate)) (Phenomenex, Le Pecq, France). The dimensions of both columns are 250 mm x 4.6 mm, 3 μm. The flow rate was 1 mL min⁻¹. All the samples were measured at wavelength 254 nm at 25°C. The optical rotation of compound 8 has been measured using KRUSS P8000 polarimeter, Optronic, Germany.

**Results and discussion**

Compounds (1-22) (Figure 1) could be classified into four groups according to the substitution at N-1 of the 2-pyrazoline ring. The first group included compounds 1-3 that contain an N-aroyl substituent. A second group comprises analytes 4-11 with arylcarboxamide moiety replacing the aroyl group. The third and fourth groups contain compounds 13-22 with alicyclic amines bound to the N-1 by either carbonyl (13-17) or methylene-carbonyl (18-22) moieties, respectively.

Normal elution mode, mixtures of alkane and lower alcohols as polar modifier (methanol, ethanol, and 2-propanol), is the recommended and most reported mobile phase for coated polysaccharide-based CSP. One elution mode is not adequate to achieve baseline separation of versatile analytes with diverse properties. Hence, the compatibility of the recently launched Lux columns (amylose-2 and cellulose-2) with all elution modes (normal, reverse, polar) provided a wider horizon for chiral separation on coated polysaccharide-based CSPs.

The exact chiral recognition mechanisms of polysaccharide-based CSPs have not been fully revealed, although expectations about formation of solute–CSP complexes through inclusion of the enantiomers into the chiral cavities of the CSP were documented.[2,3] Nonspecific interactions control the retention of the enantiomer, and stereoselective interactions regulate the separation.[1,13-16] Solute–CSP complexes are formed mainly through noncovalent molecular interactions such as hydrogen bonding, dipole–dipole, and π–π interactions between the compounds and the chiral selector groups attached to the polysaccharide.[1,15-17]

The supramolecular configuration of the polysaccharide shows dissimilar chiral cavities. The cavity of amylase is helical whereas cellulose is straight polymer chains. However, for both amylase and cellulose, the polar carbamate groups of the chiral selector are positioned inside whereas hydrophobic aromatic groups are located outside the polymer chain.[18] As well, the geometric characteristics (such as size, shape, and location of the functional groups) of the solute crucially contribute to the chiral recognition by defining the fitting extent of the solute into the chiral cavities and the strength of attractive interactions between various functional groups in the solute and the CSP.[1,16]

At the inception of the present study, the recommended mixtures of n-hexane/2-propanol or ethanol have been used to investigate the elution and separation characters of compound 4 on amylase-2 column. Using a gradient of 20–50% 2-propanol as polar modifier in n-hexane failed to give baseline separation besides late elution. The replacement of 2-propanol with ethanol led to baseline separation at 8/2 v/v within 20 min R*s = 1.54. Furthermore, the use of 50% ethanol in n-hexane enhances the resolution within shorter run time 10 min R*s = 1.77 (Table 1 and Figure 2).

### Enantioseparation using N-hexane/ethanol 1/1 v/v

Based on the efficiency of n-hexane/ethanol 1/1 v/v in separation of compound 4, this mobile phase has been selected for further investigations for separation of the 2-pyrazoline derivatives 1-22 on both amylase-2 and cellulose-2 columns. The results are summarized in Table 2.

Both amylase-2 and cellulose-2 have exhibited excellent chiral recognition abilities for all compounds except for the fourth group (compounds 19-22) on amylase-2.

It was of interest to discuss the resolution of compounds 4, 12, and 19-22. The resolution of compound 4, the most polar compound with nonsubstituted carboxamide side chain, has been tremendously enhanced from 1.71 on amylase-2 to 21.07 on cellulose-2 accompanied with delayed elution (35 min). On the other hand, compound 12, the most nonpolar with chloroacetyl side chain, showed the reverse situation being well separated on amylase-2 (R*s = 7.85) with late elution (25 min) but not separated at all on cellulose-2. Moreover, compounds 19-22 (fourth group) that have added methylene bridge compared with compounds 13-17 (third group) were fully separated on cellulose-2 with optimal R*s 8.18 but completely unresolved on amylase-2. Further substitutions in the aryl or the alicyclic amine moieties were noncrucial for the chiral recognition using n-hexane/ethanol 1/1 v/v. However, the 4-OH group has enhanced the resolution of compounds 16 and 20 on cellulose-2 column when compared to the nonsubstituted piperidine derivative 15 and 20 (Supplementary Figure 1). It is worth mentioning that cellulose-2 is better for relatively polar compounds (like 4) and amylase-2 is the best for relatively nonpolar compounds (like 12) using n-hexane/ethanol 1/1 v/v. This has been confirmed by superior resolutions of compounds 1-3 and 5-11 (less polar derivatives of 4) in comparison to 4 on amylase-2 that contrasts the obtained resolution when cellulose-2 was used. Regarding compounds 18-22, the occurrence of alicyclic amine moiety of compound 12 completely omits the recognition abilities of amylase-2 column but substantially improves the chiral recognition ability of cellulose-2 (Supplementary Figure 1). This could be explained by the decrement of lipophilicity of compound 12 —through addition of alicyclic amines—which is favored by cellulose-2 but not ideal for amylase-2 column. Concerning

### Table 1. Separation parameters of compound 4 on Lux 3 µ amylase-2 using n-hex/2-propanol or ethanol.

| Mobile phase         | t₁  | t₂  | α   | R*s |
|----------------------|-----|-----|-----|-----|
| n-Hex/2-propanol 8/2 | 27.12 | 27.93 | 1.03 | 0.57 |
| n-Hex/2-propanol 7/3 | 18.10 | 18.90 | 1.04 | 0.84 |
| n-Hex/2-propanol 1/1 | 11.65 | 12.39 | 1.06 | 1.12 |
| n-Hex/ethanol 8/2    | 18.57 | 19.79 | 1.07 | 1.54 |
| n-Hex/ethanol 1/1    | 8.16 | 8.76 | 1.07 | 1.77 |

t₁, retention time of first enantiomer; t₂, retention time of second enantiomer; α, selectivity; R*s, Resolution.
compounds 13-17, it was found that no big differences have been observed between amylose-2 and cellulose-2, but the presence of extra substituent (either polar e.g., compound 16 or nonpolar e.g., compound 17) at position 4 of the alicyclic amine improves the resolution on cellulose-2 in contrast to the use of amylose-2 (Supplementary Figure 1). These results highlight the effect of geometric characteristics in the chiral recognition abilities of polysaccharide-based CSP and could be justified by the difficult penetration of helical amylose cavity by relatively large compounds (17 and 21 vs. 16 and 20), which impairs chiral recognition. Whereas for cellulose-2, with easily penetrable supramolecular configuration, the presence of extra groups, either OH or ethyl, enables further interactions between solute and CSP that supported differentiation between enantiomers (Supplementary Figure 1).

Enantioseparation using ethanol 100%

Motivated by the role of ethanol in the enhancement of chiral recognition of amylose-2 and cellulose-2 columns and the compatibility of such columns with highly polar solvents, it was interesting to investigate the ingenuity of pure ethanol in the separation of tested compounds. Results are summarized in Table 3.

It was noticeable that the chiral recognition by both amylose-2 and cellulose-2 using 100% ethanol is very close to that of using n-hexane/ethanol 1/1 v/v. All compounds, except the fourth group (18-22) on amylose-2, have been separated within shorter time with lower resolutions but still baseline-separated. Meanwhile, cellulose-2 showed better baseline separations with extremely sharp, fast eluted peaks in comparison to amylose-2 using ethanol 100% or cellulose-2 using n-hexane/ethanol 1/1 v/v (Supplementary Figure 2). It is noteworthy that the maximum retention time for all compounds in cellulose-2 column using 100% ethanol was 12 min. Compound 4 has been eluted within 12 min and $R_s = 8.17$ (Supplementary Figure 2), which is so much better if compared to the elution on cellulose-2 using n-hexane/ethanol 1/1 v/v (35 min and $R_s = 21.07$). As well, compound 12, which was not separated on cellulose-2 using n-hexane/ethanol 1/1 v/v (Supplementary

![Figure 2. Separation of compound 4 on Lux 3 μ amylose-2 using n-hex/2-propanol or ethanol.](image-url)

| Column | Amylose-2 | Cellulose-2 |
|--------|-----------|-------------|
| Compound | $t_1$ | $t_2$ | $\alpha$ | $R_s$ | $t_1$ | $t_2$ | $\alpha$ | $R_s$ |
| 1 | 8.15 | 18.76 | 2.30 | 6.99 | 6.68 | 9.09 | 1.36 | 8.64 |
| 2 | 6.32 | 11.18 | 1.77 | 6.42 | 6.28 | 7.66 | 1.22 | 5.53 |
| 3 | 7.33 | 9.58 | 1.31 | 3.85 | 5.94 | 7.59 | 1.28 | 6.84 |
| 4 | 8.16 | 8.76 | 1.07 | 1.77 | 13.71 | 35.52 | 2.59 | 21.07 |
| 5 | 10.19 | 23.87 | 2.43 | 6.66 | 7.08 | 8.20 | 1.16 | 4.08 |
| 6 | 9.15 | 18.03 | 1.97 | 5.97 | 5.47 | 6.02 | 1.10 | 2.77 |
| 7 | 9.71 | 21.30 | 2.19 | 6.59 | 5.73 | 6.34 | 1.11 | 2.84 |
| 8 | 9.03 | 13.43 | 1.49 | 3.42 | 5.33 | 5.64 | 1.06 | 1.61 |
| 9 | 10.25 | 22.51 | 2.20 | 6.45 | 6.49 | 14.54 | 2.24 | 19.3 |
| 10 | 6.99 | 32.40 | 4.63 | 7.44 | 6.70 | 7.26 | 1.08 | 2.28 |
| 11 | 6.55 | 14.99 | 2.20 | 8.90 | 4.94 | 5.41 | 1.09 | 2.75 |
| 12 | 11.37 | 24.63 | 2.17 | 7.85 | / | / | / | / |
| 13 | 5.37 | 22.10 | 4.12 | 6.01 | 6.36 | 8.09 | 1.27 | 6.77 |
| 14 | 6.64 | 18.24 | 2.74 | 6.59 | 8.24 | 8.47 | 1.03 | 0.65 |
| 15 | 5.12 | 14.34 | 2.78 | 4.70 | 4.90 | 6.50 | 1.33 | 8.29 |
| 16 | 4.67 | 5.26 | 1.13 | 1.52 | 4.95 | 7.82 | 1.58 | 11.5 |
| 17 | 6.64 | 7.44 | 1.12 | 1.67 | 5.68 | 7.11 | 1.25 | 5.91 |
| 18 | 10.13 | 12.53 | 1.24 | 2.06 | 6.04 | 8.74 | 1.45 | 9.73 |
| 19 | / | / | / | 7.99 | 10.72 | 1.34 | 7.72 |
| 20 | / | / | / | 5.99 | 8.17 | 1.36 | 8.33 |
| 21 | / | / | / | 5.7 | 14.25 | 2.50 | 18.69 |
| 22 | / | / | / | 6.63 | 10.44 | 1.57 | 10.88 |

$t_1$, retention time of first enantiomer; $t_2$, retention time of second enantiomer; $\alpha$, selectivity; $R_s$, Resolution.
Enantioseparation using acetonitrile 100%  

The recent research reporting the significance of using acetonitrile on cellulose and amylose-based CSPs[^19-21] acted as an impetus to investigate acetonitrile efficacy in separation of compounds 1-22. Indeed, acetonitrile showed greater advantage over methanol and comparable efficacy to ethanol and n-hexane/ethanol mobile phases. In terms of peak sharpness and retention time, acetonitrile is the best mobile phase for this group of compounds using these CSPs. Using cellulose-2, baseline separations have been achieved for all compounds except 18 (Table 5 and Supplementary Figure 4).

Cellulose-2 is still sensitive to the polarity of the solute showing better recognition abilities for relatively polar compounds as seen by the resolution of compounds 1 and 12 compared to compounds 5-11 and 18-22 respectively (Supplementary Figure 4). Also, compounds 1-3 (lacking polar NH moiety) were inferiorly separated as their relatively polar analogues (compounds 5, 10, and 11 having NH group) on cellulose-2. Similarly, the third group (compounds 13-17) lacking lipophilic CH₂ moiety) was better separated on cellulose-2 than the fourth group (compounds 18-22 having the lipophilic CH₃ moiety). This was precisely the opposite when amylose-2 column was used. Further clue has been introduced by the OH group at C-4 of compounds 16 and 21, which has changed the feebly resolved (16) or unresolved (21) on amylose-2 to be fully resolved on cellulose-2 (Supplementary Figure 4). In contrary, compound 17 (with ethyl substitute in C-4) exhibited baseline separation on amylose-2, but only partial separation on cellulose-2 column has been observed (Supplementary Figure 4). Besides having very short runs, the ability of acetonitrile to resolve three members of the fourth group (compounds 18-22) on amylose-2 column represents a very important advantage over ethanol and n-hexane/ethanol systems.
Enantioseparation using acetonitrile/ethanol or methanol 1/1 V/V

To further explore the chiral recognition abilities of amylose-2 and cellulose-2 columns, a highly polar system (acetonitrile/ethanol 1/1 v/v) has been investigated for the resolution of the tested compounds. Results are summarized in Supplementary Table 1 and Figure 5.

The use of acetonitrile/ethanol as mobile phase did not introduce any added benefit over absolute ethanol or acetonitrile. It was advantageous for particular examples like Figure 3.

### Table 1

| Solvent          | Amylose | Cellulose |
|------------------|---------|-----------|
| Ethanol          | ![Chromatogram](image1) | ![Chromatogram](image2) |
| Acetonitrile     | ![Chromatogram](image3) | ![Chromatogram](image4) |
| Methanol         | ![Chromatogram](image5) | ![Chromatogram](image6) |
| Acetonitrile/ethanol | ![Chromatogram](image7) | ![Chromatogram](image8) |
| Acetonitrile/methanol | ![Chromatogram](image9) | ![Chromatogram](image10) |
| Hex/ethanol      | ![Chromatogram](image11) | ![Chromatogram](image12) |

**Figure 3.** Separation chromatograms of compound 8 on Lux 3 μ amylose-2 and cellulose-2 using different mobile phases showing reversal of elution order.
compound 4, which has been baseline-separated within 6 min with $R_s = 7.16$ on cellulose-2. With regards to run time, acetonitrile/ethanol 1/1 in $v/v$ has achieved great improvement over 100% acetonitrile (36 min), 100% ethanol (12 min), and $n$-hexane/ethanol (35 min) for compound 4. The preference of cellulose-2 column toward more polar compounds has been changed with respect to third and fourth groups. Thus, using this solvent combination, compounds (18-22) were more efficiently resolved on cellulose-2 column than in compounds 13-17. However, in the case of amylose-2 column the situation was still similar to absolute ethanol or acetonitrile.

With respect to the number of compounds that were successfully baseline-separated, acetonitrile/methanol system showed superiority to acetonitrile/ethanol. Also, amylose-2 displayed greater chiral recognition abilities than cellulose-2 column using such highly polar combinations (Supplementary Table 2 and Figure 6).

### Elution order in polysaccharides-based CSP

It has been previously documented that the elution order is reversible while altering between amylose and cellulose-based CSP[22–25]. More recently, other factors like additives to the mobile or stationary phases led to varied affinity patterns of compound 8.

Since the detection was not based on optical rotation. The only reported justification for reversed elution order was the change in supramolecular configuration of the CSPs that could result from varying the elution mode. Occasionally, reverse order of elution happens when changing the mobile phase on the same CSP[29] as well as using the same mobile phase but changing the CSP.[30] In the current study, both cases have been reported since the elution order of compound 8 was reversed when changing between amylose-2 and cellulose-2 column under all polar organic mode mobile phases. As well, when changing the elution mode from polar organic to normal on cellulose-2 the elution order has been reversed (Figure 3).

### Conclusions

The polysaccharide-phenylcarbamate-based CSPs, Lux amylose-2 and cellulose-2, have exhibited outstanding chiral recognition abilities toward different bioactive chiral 2-pyrazolines 1-22 in both polar organic and normal elution modes. Very high resolutions were realized for most compounds under different elution modes. Mechanistic approach based on different interactions between the CSP and compounds 1-22 and its effects on the chiral recognition abilities of Lux columns have been discussed. It has been confirmed that the intermolecular forces involved in analyte retention and enantioseparation are diverse. Thus, both the mobile and stationary phases, as well as the nature of the analyte, are contributing to the chiral recognition. Several chiral analytical methods for compounds 1-22 have been introduced and they can be utilized for monitoring asymmetric reactions or optimizing preparative chiral HPLC resolutions. Polar organic mode (acetonitrile 100%) was suggested for reaction monitoring since it showed optimum resolution within short analysis times and favorable peak shape. Fast elution is very crucial when having several samples to be analyzed as in quality control work, reactions follow-up, or pharmacological and toxicological screening. Normal mode ($n$-hexane/ethanol) was instructed for the development of preparative HPLC methods due to the big difference in retention between two enantiomers that will support column loadability. Altering the mobile or the stationary phases led to varied affinity patterns of compound 8.

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### References

[1] Okamoto, Y.; Yashima, E. Polysaccharide Derivatives for Chromatographic Separation of Enantiomers. *Angew. Chem. Int. Ed.* 1998, 37 (8), 1020–1043

[2] Shen, J.; Okamoto, Y. Efficient Separation of Enantiomers Using Stereoregular Chiral Polymers. *Chem. Rev.* 2016, 116 (3), 1094–1138.

[3] Lorenz, H.; Seidel-Morgenstern, A. Processes to Separate Enantiomers. *Angew. Chem. Int. Ed.* 2014, 53 (5), 1218–1250.
[4] Francotte, E. R. Enantioselective Chromatography as a Powerful Alternative for the Preparation of Drug Enantiomers. *J. Chromatogr. A* 2001, 906 (1–2), 379–397.

[5] Özdemir, A.; Altıntop, M. D.; Kaplançıklı, Z. A.; Turan-Zitouni, G.; Çiçek, G. A.; Yıldırım, Ş. U. Synthesis of 1-acetyl-3-(2-thienyl)-5-aryl-2-pyrazoline Derivatives and Evaluation of Their Anticancer Activity. *J. Enzyme Inhib. Med. Chem.* 2013, 28 (6), 1221–1227.

[6] Carradori, S.; Secci, D.; Bolasco, A.; De Monte, C.; Yáñez, M. Synthesis and Selective Inhibitory Activity Against Human COX-1 of Novel 1-(4-Substituted-thiazol-2-yl)-3,5-di(hetero)aryl-pyrazoline Derivatives. *Arch. Pharm.* 2012, 345 (12), 973–979.

[7] Cottineau, B.; Toto, P.; Marot, C.; Pipaud, A.; Chenault, J. Synthesis and Hypoglycemic Evaluation of Substituted Pyrazole-4-carboxylic Acids. *Bioorg. Med. Chem. Lett.* 2002, 12 (16), 2105–2108.

[8] Rostom, S. A.; Shalaby, M. A.; El-Demellawy, M. A. Polysubstituted Pyrazoles, Part 5. Synthesis of New 1-(4-Chlorophenyl)-4-hydroxy-1H-pyrazole-3-carboxylic Acid Hydrazide Analogs and Some Derived Ring Systems. A Novel Class of Potential Antitumor and Anti-HCV Agents. *Eur. J. Med. Chem.* 2003, 38 (11–12), 959–974.

[9] Aboul-Enein, M. N.; El-Azzouny, A. A.; Attia, M. I.; Maklad, Y. A.; Rostom, S. A.; Shalaby, M. A.; El-Demellawy, M. A. Polysubstituted Pyrazoles. Synthesis of Novel Stipentanol Analogues as Potential Anticonvulsants. *Eur. J. Med. Chem.* 2012, 47, 360–369.

[10] Chakrabarti, J. K.; Eggleton, R. I.; Gallagher, P. T.; Harvey, J.; Hicks, T. A.; Kitchen, E. A.; Smith, C. W. 5-Acyl-3-substituted-benzofuran-2(3H)-ones as Potential Antiinflammatory Agents. *J. Med. Chem.* 1987, 30 (9), 1663–1668.

[11] El-Behairy, M. F.; Mazeed, T. E.; El-Azzouny, A. A.; Aboul-Enein, M. N. Design, Synthesis and Antibacterial Potential of 5-(benzo[d][1,3]dioxol-5-yl)-3-tert-butyl-1-substituted-4,5-dihydropyrazoles. *Saud J. Pharm. J.* 2015, 23 (2), 202–209.

[12] Akbas, E.; Berber, I. Antibacterial and Antifungal Activities of New pyrazolo[3,4-d]pyridazin Derivatives. *Eur. J. Med. Chem.* 2005, 40 (4), 401–405.

[13] Cirilli, R.; Ferretti, R.; Gallinella, B.; Turchetto, L.; Bolasco, A.; Secci, D.; Chimenti, P.; Pierini, M.; Fares, V.; Befani, O.; La Torre, F. Enantiomers of C5-chiral 1-acetyl-3,5-diphenyl-4,5-dihydro-(1H)-pyrazole Derivatives: Analytical and Semi-preparative HPLC Separation, Chiroptical Properties, Absolute Configuration, and Inhibitory Activity Against Monoamine Oxidase. *Chirality* 2004, 16 (9), 625–636.

[14] Lämmerhofer, M. Chiral Recognition by Enantioselective Liquid Chromatography: Mechanisms and Modern Chiral Stationary Phases. *J. Chromatogr. A* 2010, 1217 (6), 814–856.

[15] Okamoto, Y.; Kaida, Y. Resolution by High-performance Liquid Chromatography Using Polysaccharide Carabamates and Benzoxates as Chiral Stationary Phases. *J. Chromatogr. A* 1994, 666 (1–2), 403–419

[16] Wang, T.; Wenslow, R. M. Effects of Alcohol Mobile-phase Modifiers on the Structure and Chiral Selectivity of Amylose tris (3,5-dimethylphenylcarbamate) Chiral Stationary Phase. *J. Chromatogr. A* 2003, 1015 (1–2), 99–110.

[17] Aboul-Enein, H. Y.; Ali, I. Optimization Strategies for HPLC Enantioseparation of Racemic Drugs Using Polysaccharides and Macrocyclic Glycopeptide Antibiotic Chiral Stationary Phases. *Il Farmaco* 2002, 57 (7), 513–529.

[18] Winger, M.; Christen, M.; van Gunsteren, W. F. On the Conformational Properties of Amylose and Cellulose Oligomers in Solution. *Int. J. Carbonyl. Chem.* 2009, 2009, 1–8.

[19] Gogaladze, K.; Chankvetadze, L.; Tsintsadze, M.; Farkas, T.; Chankvetadze, B. Effect of Basic and Acidic Additives on the Separation of Some Basic Drug Enantiomers on Polysaccharide-Based Chiral Columns with Acetonitrile as Mobile Phase. *Chirality* 2015, 27 (3), 228–234.

[20] Sharma, P.; Contractor, P.; Guttiar, S.; Patel, D. P.; Shrivastav, P. S. Development of a Sensitive and Rapid Method for Quantitation of (S)-(−) and (R)-(+) -metopol in Human Plasma by Chiral LC–ESI–MS/MS. *J. Pharm. Anal.* 2014, 4 (1), 63–79.

[21] Zhang, H.; Qian, M.; Wang, X.; Wang, X.; Xu, H.; Wang, Q.; Wang, M. HPLC-MS/MS Enantioseparation of Triazole Fungicides Using Polysaccharide-based Stationary Phases. *J. Sep. Sci.* 2012, 35 (7), 773–777.

[22] Ma, S.; Shen, S.; Lee, H.; Eriksson, M.; Zeng, X.; Xu, J.; Fandrick, K.; Yee, N.; Senanyake, C.; Grinberg, N. Mechanistic Studies on the Chiral Recognition of Polysaccharide-based Chiral Stationary Phases Using Liquid Chromatography and Vibrational Circular Dichroism. *J. Chromatogr. A* 2009, 1216 (18), 3784–3793.

[23] Chankvetadze, B.; Yamamoto, C.; Okamoto, Y. Enantioseparation of Selected Chiral Sulfoxides Using Polysaccharide-type Chiral Stationary Phases and Polar Organic, Polar Aqueous–organic and Normal-phase Eluents. *J. Chromatogr. A* 2001, 922 (1–2), 127–137.

[24] Ali, I.; Naim, L.; Gharem, A.; Aboulenein, H. Chiral Separations of Piperidine-2,6-dione Analogues on Chiralpak IA and Chiralpak IB Columns by Using HPLC. *Talanta* 2006, 69 (4), 1013–1017.

[25] Chankvetadze, B. Recent Developments on Polysaccharide-based Chiral Stationary Phases for Liquid-phase Separation of Enantiomers. *J. Chromatogr. A* 2012, 1269, 26–51.

[26] Mosialovili, L.; Chankvetadze, L.; Farkas, T.; Chankvetadze, B. On the Effect of Basic and Acidic Additives on the Separation of Some Basic Drugs with Polysaccharide-based Chiral Selectors and Polar Organic Mobile Phases. *J. Chromatogr. A* 2013, 1317, 167–174.

[27] Yang, X.; Su, L.; Hou, X.; Ding, S.; Xu, W.; Wang, B.; Fang, H. High-performance Liquid Chromatographic Enantiomeric Separation of 3,5-disubstituted Hydantoin Analogs and Temperature-induced Reversals of Elution Orders on a Polysaccharide-based Chiral Stationary Phase. *J. Chromatogr. A* 2014, 1355, 291–295.

[28] Gharem, A. True and False Reversal of the Elution Order of Barbiturates on a Bonded Cellulosic-based Chiral Stationary Phase. *J. Chromatogr. A* 2006, 1132 (1–2), 329–332.

[29] Aboul-Enein, H. Y.; Ali, I. Studies on the Effect of Alcohols on the Chiral Discrimination Mechanisms of Amylose Stationary Phase on the Enantioseparation of Nebivolol by HPLC. *J. Biochem. Biophys. Methods* 2001, 48 (2), 175–188.

[30] Bonato, P. S.; de Abreu, L. R.; de Gaitani, C. M.; Lanchote, V. L.; Bertucci, C. Enantioselective HPLC Analysis of Propafenone and Its Main Metabolites Using Polysaccharide and Protein-based Chiral Stationary Phases. *Biomed. Chromatogr.* 2000, 14 (4), 227–233.