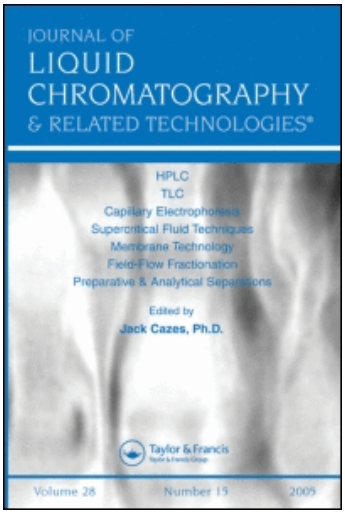



**Journal of Liquid Chromatography & Related Technologies**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

# Separation of Benzodioxinic Isomers in LC. A Molecular Modeling Approach for the Choice of the Stationary Phase

K. Azzaoui[a]; M. Lafosse[a]; S. Lazar[a]; V. Thiéry[a]; L. Morin-Allory[a]
[a] Laboratoire de Chimie Bioorganique et Analytique URA CNRS 499, Université d'Orléans, Orléans, Cedex, France

**To cite this Article** Azzaoui, K. , Lafosse, M. , Lazar, S. , Thiéry, V. and Morin-Allory, L.(1995) 'Separation of Benzodioxinic Isomers in LC. A Molecular Modeling Approach for the Choice of the Stationary Phase', Journal of Liquid Chromatography & Related Technologies, 18: 15, 3021 — 3034
**To link to this Article: DOI:** 10.1080/10826079508010430
**URL:** http://dx.doi.org/10.1080/10826079508010430

JOURNAL OF LIQUID CHROMATOGRAPHY, 18(15), 3021-3034 (1995)

# SEPARATION OF BENZODIOXINIC ISOMERS IN LC. A MOLECULAR MODELING APPROACH FOR THE CHOICE OF THE STATIONARY PHASE

**K. AZZAOUI, M. LAFOSSE, S. LAZAR, V. THIÉRY, AND L. MORIN-ALLORY***
*Laboratoire de Chimie Bioorganique et Analytique*
*URA CNRS 499*
*Université d'Orléans*
*B.P. 6759. 45067 Orléans Cedex 2, France*



## ABSTRACT

Seven couples of position isomers on the aromatic ring of benzodioxinic compounds had to be separated. The tries made on classical systems in gas chromatography or liquid chromatography did not allow the separation. We have studied the structures of solutes by molecular modeling. Many molecular descriptors (lipophilic, electronic and steric) were calculated. Our objective was to compare the relative difference between the isomers in term of lipophilicity, electronic and steric properties and design the chromatographic system where these interactions are dominant. From these results, the porous graphitic carbon stationary phase was chosen and allowed a good separation of all the products.

## INTRODUCTION

As part of our search for antiatherosclerotic agents [1,2] we needed 2-substituted-2,3-dihydro-1,4-benzodioxin (Fig. 1a) and 2-substituted-1,4-

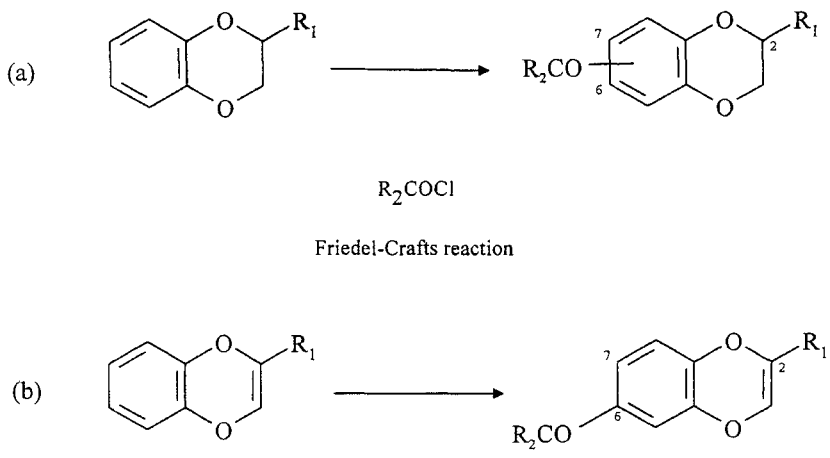



FIGURE 1 : The Friedel-Crafts acylation of Benzodixanic compounds.



benzodioxin (Fig. 1b) derivatives with acylated group on several positions of the aromatic ring [3]. The Friedel-Crafts acylation of saturated derivatives leads to an isomeric mixture of two monoacylated products at $C_6$ and $C_7$ positions with the 2,7 isomer as the main product (Fig. 1a). In contrast the same reaction using unsaturated precursors yields regioselectively the $C_6$ acetylated derivative (Fig. 1b).

In order to compare the reactivity depending on the substitution position, we converted the saturated isomeric mixture (Fig. 1a with $R_2 = CH_3$ and $R_1 = CN$) in a few steps to the 2,6 and 2,7 unsaturated analogues [3].

We used chromatographic analysis to study the regioselectivity of these reactions of acylation and to determine the isomeric ratio of each isomer identified by $^1$H-NMR.

After various unsuccessful attempts on GC columns (no selectivity or no elution) and on LC columns (with bare silica in normal phase and octadecyl silica in reversed phase) we used molecular modeling to understand the retention of model compounds ($R_1$=CN). The results of this theoretical study allow us to

explain the difficulty in separating of these isomers on classical ODS stationary phase and the choice of porous graphitic carbon phase (PGC) to separate them.

The PGC column possesses a rigid, planar surface in addition to high electronic and charge transfer interactions and this is an advantage for the separation of our isomeric compounds [5].

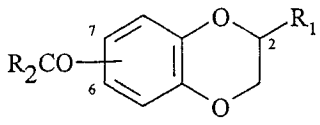
## EXPERIMENTAL

### 1- Materials

The HPLC equipment comprised the following components: Gilson Model 302 pump (Villiers le Bel, France) and Rheodyne Model 7125 injection valve (Cotati, California, U.S.A); for UV detection at 254 nm, a Jasco Model 2550 spectrophotometer (Tokyo, Japan). Quasi-identical UV response of isomers at this wavelength was confirmed by an evaporative light scattering detector Model Sedex(ELS) 45 (Sedere, France) set on line. In fact, the narrow spread of the response factors of the ELS detector indicated that it is suitable for direct raw quantification [6]. Data were processed with a Shimadzu model CR 5A integrator/recorder (Kyoto, Japan).

HPLC analyses were carried out in normal phase on 250 x 4.6 mm i.d. column packed with 7 µm Zorbax Sil (DuPont, Wilmington, U.S.A); in reverse phase on 125 x 4 mm i.d. column packed with 5 µm Lichrospher 100 RP18 (Merck, Darmstadt, Germany); with various mobile phases on 150 x 4.6 mm i.d. column packed with Hypercarb (Shandon, Cergy-Pontoise, France). The first two systems do not give any selectivity between isomers.

Experiments by Gas Chromatography were carried out using a Varian GC Model 3700 (Les Ulis, France) on various columns: 3 m, 0.2% Silar 10C coated on 80/100 Graphpac-GC (Alltech, Templeuve, France) in packed glass column; 25 m 1 µm, 530 µm o.d., BP-1 fused silica column and 12 m 0.5 µm, 530 µm o.d., BPX70 fused silica column (SGE, Villeneuve St. Georges, France). On the first




| Compounds | $R_1$ | $R_2$ |
|---|---|---|
| 1 | CN | CH3 |
| 2 | CN | $(CH2)_3CH_3$ |
| 3 | $CO_2H$ | CH3 |
| 4 | $CH_2COCH_3$ | CH3 |
| 5 | $CH_2OCO(CH_2)_2CH_3$ | $(CH2)_3CH_3$ |
| 6 | $CH_2OCOCH_3$ | $(CH2)_3CH_3$ |



**Compounds 7**

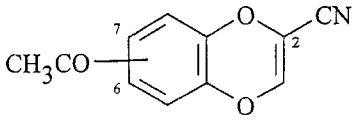



FIGURE 2 : The list of compounds.

column high adsorption of solutes precludes their elution. On both capillary columns no selectivity is obtained.

## 2- Mobile Phase and Reagents

The mobile phases, acetonitrile, methanol, water, methylene chloride (HPLC grade) and trifluoroacetic acid were obtained from Aldrich (St Quentin Fallavier, France).

Compounds 1-7 ( Fig 2) were synthesised according to Thiéry *et al.* [3] by treatment of 2-substituted 2, 3 dihydro-1.4 benzodioxin with various acylchlorides

in presence of aluminium trichloride and carbon disulfide. In order to determine unambiguously the right position of the acetyl group, we prepared on the basis of literature data [4] and isolated in the pure form the 2,6 and 2,7 unsaturated derivatives.

## 3- Molecular modeling

We studied only the products substituted with CN group in position 2 and acetyl group in position 6 or 7 with or without the double bond (compounds 1 and 7). These model compounds were the easiest to study due to the small number of stable conformers.

Molecular structures were constructed from the input operating mode of the molecular modeling program Macromodel V3.5X [7] on Silicon Graphics Iris.

We carried out a systematic conformational search (MULTIC program) using the MM2 force field [8] with parametrisation for a isolated compound. All the conformations with good convergence were kept.

The molecular geometries of these conformations were optimised using the semi-empirical orbital program Mopac5 [9] (key-words: *am1, precise, polar*).

For each conformer i we calculated several descriptors $D_i$. The descriptor value D for a compound is then obtained from the values of this descriptor for all the stable conformations using equations 1 and 2.

For a descriptor value D and a conformation i (equation 1):

$$D = \sum_i Fi \times Di$$

where $F_i$ is the molar fraction obtained from the Boltzman distribution at 25 °C (equation 2):

$$Fi = \frac{e^{-\Delta Ei/RT}}{\sum_i e^{-\Delta Ei/RT}}$$

$\Delta E_i$ is the energy difference between the conformer i and the most stable conformer.

We chose descriptors which have been used with success in other QSRR studies and also some other descriptors. These descriptors are:

*surf* and *vol*: respectively the Connolly surface and volume [10] (solvent accessible surface and volume) generated by program Molcad [11]. Specific properties such as molecular potential (*mep* or *mlp*) can be mapped on the Connolly surface.

*mep* : the molecular electrostatic potential [12] calculated on the Connolly surface.

*mlp* : the molecular lipophilic potential of the structure calculated on the Connolly surface. This potential is calculated using the Fauchère *et al* formula [13] and Ghose-Crippen's [14] atomic contribution.

The integrated value (*mepint* or *mlpint* ) [15] is obtained by the algebraic sum on all the points computed by the Molcad program with the default density of the Connolly surface. This descriptor is therefore not only an electronic or lipophilic but also a geometric one.

*oval* : ovality (defined as the ratio of the actual surface area and its minimum surface area).

*mtdip* : the total dipole moment of the structure.

*pol* : the molecular polarisability calculated in an electric field.

*homo* : the energy level of the highest occupied molecular orbital.

*lumo* : the energy level of the lowest unoccupied molecular orbital.



## RESULTS AND DISCUSSION

### 1- Quantitative Structure-Retention Relationships.

#### 1-1 Molecular Modeling Study

In Table 1, we present the descriptor values of the two isomers (6-acetyl and 7-acetyl) of saturated compound 1. Each isomer has four stable conformers

## Table 1

### The calculated descriptors for isomers of compound 1.

| | *pol* | *mf dip* | *pimint* | *pemint* | *surf* | *vol* | *oval* | *homo* | *lumo* |
|---|---|---|---|---|---|---|---|---|---|
| **D 2-6** | 16.360 | 3.680 | 7.429 | -485.491 | 214.829 | 204.867 | 1.279 | -9.520 | -0.628 |
| **D 2-7** | 16.390 | 3.615 | 7.445 | -464.264 | 214.911 | 204.832 | 1.279 | -9.518 | -0.649 |
| **rd** | 0.030 | 0.066 | 0.016 | 21.227 | 0.083 | 0.035 | 0.001 | 0.002 | 0.021 |
| **% rd** | 0.16 | 1.78 | 0.21 | 4.37 | 0.04 | 0.02 | 0.05 | 0.02 | 3.38 |

**D 2-6** : the descriptor value for isomer with acetyl in position 6.

**D 2-7** : the descriptor value for isomer with acetyl in position 7.

**rd** : the absolute value of the difference on descriptors between the two isomers.

**% rd** : the relative difference value in percentage.

(two axial and two equatorial). The CN axial conformers are energetically the most favourable.

For each isomer, we calculated the value of each descriptor (according to equations 1 and 2). We also calculated the difference (**rd**) and the relative difference (**%rd**) between the two descriptors of 6-acetyl and 7-acetyl isomers.

The greater **%rd** values were noticed for *pemint* (4.37%), *lumo* (3.38%) and *mtdip* (1.78%). The others **%rd** are less than 0.21% (*pol, plmint, surf, vol* and *homo*).

In Table 2, we present the descriptor values of the two isomers (6-acetyl and 7-acetyl) of unsaturated compound 7. Due to the presence of the double bond, each isomer has only two stable conformers. We calculated **D**, **rd** and **%rd** as above. The main difference of this compound *vs* the compound 1 is its global shape. All the atoms of the rings and substituents (CN and acetyl) lie into a plane.

For compound 7, the greater **%rd** values were noticed for *mtdip* (14.3%), *pemint* (10.8%). The others **%rd** are less than 3% (*pol, plmint, surf, vol, oval, homo* and *lumo*).

For the calculation of lipophilic descriptors we do not use log P (calculated partition coefficient in octanol/water system using fragmental approach) as lipophilic descriptor because two position isomers have the same calculated log P. The integrated molecular lipophilic potential (*plmint*) calculated on Connolly surface is a lipophilic but also a geometric descriptor, consequently the *plmint* of two isomers may be different.

The difference between isomers in *plmint* (lipophilic descriptor), molecular volume and molecular surface ('bulk' descriptors) is very low. The difference is much greater for electronic descriptors like dipole moment and the integrated molecular electrostatic potential.

## 1-2 Correlation with Chromatographic Retention

When we tried to separate the isomers on apolar stationary phases ( ODS and BP1) and polar stationary phases (silica and BPX70) in liquid and gas

## Table 2

### The calculated descriptors for isomers of compound 7.

| | *pol* | *mt dip* | *pimint* | *pemint* | *surf* | *vol* | *oval* | *homo* | *lumo* |
|---|---|---|---|---|---|---|---|---|---|
| **D 2-6** | 16.883 | 2.311 | 1.957 | -364.852 | 209.724 | 196.047 | 1.285 | -9.199 | -1.014 |
| **D 2-7** | 16.955 | 2.643 | 2.007 | -325.552 | 209.743 | 196.365 | 1.284 | -9.203 | -0.988 |
| **rd** | 0.072 | 0.331 | 0.050 | 39.300 | 0.019 | 0.318 | 0.001 | 0.003 | 0.026 |
| **% rd** | 0.4 | 14.3 | 2.6 | 10.8 | 0.0 | 0.2 | 0.1 | 0.0 | 2.6 |

**D 2-6** : the descriptor value for isomer with acetyl in position 6.

**D 2-7** : the descriptor value for isomer with acetyl in position 7.

**rd** : the absolute value of the difference on descriptors between the two isomers.

**% rd** : the relative difference value in percentage.

chromatography respectively, no selectivity was obtained. These results can be explained by the small differences between the lipophilic descriptors of the isomers.

Recently, Forgacs *et al* [16] studied the retention of 22 phenol derivatives on porous graphitized carbon (PGC) and octadecylsilica (ODS). They evaluated the relationships between retention and physicochemical parameters by multivariate analysis. Calculations proved that a marked difference can be detected between the retention characteristics of PGC and ODS columns, and that the electronic parameters of phenol derivatives have the highest impact on their retention.

Kaliszan *et al* [17] have used quantitative structure-retention relationships (QSRR) to analyse HPLC retention data for a set of non-congeneric aromatic solutes. They used graphitic carbon as stationary phase with hexane as eluent. The capacity factors were quantitatively related to structural information extracted from nineteen molecular descriptors of solutes by multivariate analysis. These QSRR studies using principal components provide evidence for the decisive role of specific, polar electronic interactions for the separation of solutes in PGC-hexane HPLC system.



The electronic descriptors are the ones with the greatest difference for the couple of isomers of our compounds (*mtdip* and *pemint* mainly).

According to these results, we tried to separate the isomers on Hypercarb column. The mobile phase used depends on the class of benzodioxinic compounds to be separated, saturated or unsaturated ones.

With a benzodioxinic saturated compound like solute 1, a better selectivity is obtained with acetonitrile-water-trifluoroacetic acid (57:42.7:0.3 %) as mobile phase. Using this mobile phase, unsaturated CN-substituted compounds as solute 7 are not eluted, they require a pure organic mobile phase. It is can be explained by the difference in the adsorption energy on the PGC planar surface and the surface of the adsorbed molecule. Compound 7 is much more planar than compound 1 thus it has a high interaction with PGC surface.

This qualitative study shows that we can design a better system to separate a set of compounds using molecular modeling and theoretical properties calculations.

2- Chromatographic Results

For the compounds having a moderately polar substituent such as $R_1 = CH_2OCOCH_3$ and $R_2 = CH_3$ (pair of solutes 4), a good selectivity is obtained on Hypercarb using an acetonitrile-water 85:15 (v/v) mixture as mobile phase, this packing being used as a "pure" reversed phase material (Chromatogram 4, Fig. 3).

When $R_1 = CH_2OCOCH_3$ and $R_2 = (CH_2)_2CH_3$ (pair of solutes 6), the retention increases considerably with such a mobile phase. For sufficiently selective elution of this pair of isomers, plain acetonitrile as mobile phase is required (Table 3; Chromatogram 6, Fig. 3). With the same eluent, isomers with $R_1 = CH_2OCO(CH_2)_2CH_3$ and $R_2 = (CH_2)_2CH_3$ (pair of solutes 5, Fig. 3) are more retained.

When $R_1$ substituent is more polar (CN, COOH) (pair of solutes 1 and 3 respectively), an electronic modifier (e.g. trifluoro acetic acid) is required to obtain a good shape of peak and to avoid peak tailing. In fact this is due to the presence of an electron pair of trifluoroacetic acid which acts as an electron donor and decreases the charge transfer between analyte and porous graphitic carbon. With 42.7 % of water content in the mobile phase, the pair of solutes 1 are *separated (Table* 3, *Fig.* 3), in retention time of 6.2 min. Using this mobile phase, unsaturated CN-substituted compounds are not eluted, showing the large difference between these two series.

To increase the hydrophobic character of CN-substituted isomers, $R_2 = (CH_2)_3CH_3$ (pair of solutes 2) replaced $R_2 = CH_3$ (solutes 1). Consequently a larger amount of organic modifier (acetonitrile) is required to elute the isomers with similar retention (Chromatogram 2, Fig. 3).

When the substituent is a polar group such as COOH (solutes 3), a better selectivity is obtained with a free-water mobile phase (Table 3; chromatogram 3,

Table 3

Selectivity of the separation of isomers.

| Isomers mixture | α | Mobile phase |
|---|---|---|
| 1 | 1.16 | CH3CN-H2O-CF3COOH (57/42.7/0.3) |
| 2 | 1.34 | CH3CN-H2O-CF3COOH (200/50/1) |
| 3 | 1.15 | CH3CN-H2O (100/1) |
| 4 | 1.25 | CH3CN-H2O (85/15) |
| 5 | 1.21 | CH3CN |
| 6 | 1.15 | CH3CN |
| 7 | 1.37 | CH2Cl2 |



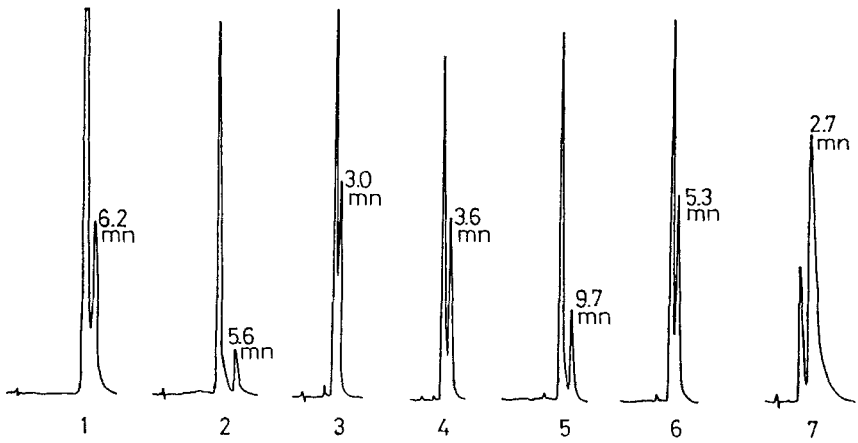



FIGURE 3 : The chromatograms of the separated isomers.

Fig. 3) and the comparison of the retention with that of the corresponding CN-substituted compound demonstrates the more hydrophobic character of the first derivative.

With the benzodioxinic derivatives where $R_1$ = CN and $R_2$ = $CH_3$ (solutes 7), the stronger interactions between solutes and porous graphitic carbon surface require a pure organic mobile phase (methylene chloride) for elution (Chromatogram 7, Fig. 3). Such a mobile phase is required by the strong adsorption potential of this material with these flat molecules. This illustrates the large choice of solvents used with porous graphitic carbon. Comparison of the behaviour of saturated and unsaturated CN-substituted compounds illustrates the great influence of the molecule's stereochemistry in the process of separation and we can note that for saturated compounds, the 2-7 isomer (the higher peak in chromatograms 1-6, Fig. 3) is less retained when for unsaturated ones the 2-7 isomer (the higher peak in chromatogram 7, Fig. 3) is more retained.



## CONCLUSION

In this work, we have used molecular modeling to design a better chromatographic system to separate isomers. The Hypercarb was the best stationary phase used for the separation of these isomers. We have explained the separation on the PGC stationary phase by the large relative difference in the electronic properties of the isomers compared to their lipophilic ones.

This is the proof of the usefulness of molecular modeling in the rational choice of a chromatographic system.

## ACKNOWLEDGEMENTS

The authors gratefully acknowledge F. Villard for his technical assistance and Shandon company for the loan of Hypercarb column.

Present address of S. Lazar: Faculté des Sciences et Techniques. BP 146. Mohammedia. Maroc.

## REFERENCES

1. T. Yoshioka, T. Fujita, T. Kanai, Y. Aizawa, T. Kurumada, K. Hasegawa and H. Horikoshi, J. Med. Chem., 32: 421 (1989).

2. Y. Aizawa, T. Kanai, K. Hasegawa, T. Yamaguchi, Y. Lizuka, T. Iwaoka and T. Yoshioka, J. Med. Chem., 33: 1491 (1990) .

3. V. Thiéry, G. Coudert, L. Morin-Allory and G. Guillaumet, Tetrahedron, to be published.

4. S.F. Campbell, M.J. Davey, J.D. Hardstone and B.N. Lewis, J. Med. Chem., 30: 49 (1987).

5. M. Josefsson, B. Carlsson and B. Norlander, Chromatographia., 37: 129 (1993).

6. M. Lafosse, C. Elfakir, L. Morin-Allory and M. Dreux, HRC., 15: 312 (1992).

7. F. Mohamadi, N. G. J. Richards, W.C. Guida, R. Liskamp, C. Caufield, G. Chang, T. Hendrickson, W. C. Still, J. Comput. Chem., 11: 440 (1990).

8. N. L. Allinger, J. Am. Chem. Soc., 99: 1579 (1977).

9. J. J. P. Stewart. J. Comput.-Aided Mol. Design., 4: 1 (1990).

10. M. L. Connolly, Science., 211: 709 (1983).

11. W. Heiden, M. Schenkrich and J. Brickmann, J. Comput.-Aided Mol. Design., 4: 255 (1990).

12. P. K. Weiner, R. Langridge, J. M. Blaney, R. Schaefer and P. A. Kollman, Proc. Natl. Acad. Sci. U. S. A., 79: 3754 (1982).

13. J.- L. Fauchère, P. Quarnedon and L. Kaetterer, J. Mol. Graph., 6: 203 (1988).

14. A. K. Ghose and G. M. Crippen, J. Comput. Chem., 7: 565 (1986).

15. L. Morin-Allory, Unpublished results.

16. E. Forgacs, T. Cserhati, B. Bordas, Chromatographia., 36: 19 (1993).

17. R. Kalizsan, K. Osmialowski, B. J. Bassler and R. A. Hartwick, J. Chromatogr., 499: 333 (1990).

Received: February 18, 1995
Accepted: May 9, 1995