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Separation of Racemates Using Chiral HPLC and Creation of a Database for this Methodology

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The selection of an appropriate system such as chiral stationary phase (CSP) and operating condition often remains difficult to find by trial and error. A database was initiated in HPLC to fill this gap. Two databases software were readily available as possible tools for achieving the objectives of the project. First, ISIS base was considered initially as a suitable tool for the project but the shortcoming was the disability to store chromatograms. Second, Microsoft Access was considered as an alternative, but it was unable to handle chemical information. So a secondary software called Accord for Microsoft Access was added to give chemical awareness. With the Accord add-in great chemical applications can create directly within Microsoft Access 97's. It gives a complete picture of the analyte including the chromatogram. The database was fed by information about the separation of twenty eight enantiomers and the separations have been carried out using the five chiral stationary phases (CSPs) available in the chemistry department at University of Manchester Institute of Science and Technology (UMIST). The database was examined for selection of a suitable column and the suitable conditions for chiral separation of enantiomers and it gave a successful and effective conclusion. Therefore this developed method save time, materials used for finding out the optimum conditions and labour.

Key Words: Separation of racemates, Chiral HPLC, Databases.

INTRODUCTION

Virtually every area of modern life has been touched by computer technology. In many instances this has resulted in profound changes in our daily activities. Although these changes are already taken for granted by many, it has only been in the last 10-20 years that the exponential growth of computer applications has occurred. Benefits of computerization have long been recognized by users of analytical instruments; however, only recently have the capabilities and available software for computers been at such a

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level that they can be used effectively by non-programmers. Chirality has become increasingly important in pharmaceutical, chemical and agriculture activities. Enantiomers of pharmaceutical compounds may display quite different pharmacological behaviours[1].

Various types of chiral stationary phases have been developed for chromatography. Several publications[2,3], review articles[4,5] and research papers[6,7] describe the basis of separation on different columns that are be suited to certain types of analytes. Among the various chiral stationary phases (CSPs), cellulose and amylose based CSPs have been proved to be quite versatile. A wide variety of enantiomeric compounds, including chiral aromatic alcohols[8], enantiomeric amides[9,10], amino alcohols[11], diols[12] and racemic carboxylic acids[13] have been separated on these CSPs. Therefore, the development of analytical methods that can separate and quantify the enantiomers plays a very important role in the drug development process. The most popular technique used for separation and quantification of the enantiomers is high-performance liquid chromatography (HPLC). In the development of a HPLC method, it is usually desirable to use a chiral stationary phase (CSP) to directly separate the enantiomers because of the simplicity and ease of operation related to this approach. Since there are so many CSPs available to end-user[2] including Pirkle type phases[14], chiral cavity phases[15-17], helical polymer phases[18] the selection of appropriate system (CSP and operating conditions) often remains difficult to find by trial and error and might be highly expensive in terms of time, material and labour. In this paper the CHIRAL-UMIST database was initiated in HPLC in the chemistry department at University of Manchester Institute of Science and Technology (UMIST) to fill this gap. Data include ID, by-whom, date-enter, sample name, stationary phase, structure, chromatogram, mobile-phase, flow-rate, temperature, wavelength, Rt_1, Rt_2, k_1, k_2, resolution and selectivity which gave a complete picture of the separation. Twenty-eight pairs of enantiomers were separated using the five chiral stationary phases (CSPs) available in the chemistry department at (UMIST). These chiral stationary phases were amylose *tris*(3,5-dimethylphenylcarbamate) (Chiralpak AD), cellulose *tris*(3,5-dimethylphenylcarbamate) (Chiralcel OD), amylose *tris*[(S)-1-phenylethylcarbamate] (Chiralpak AS), cellulose tribenzoate (Chiralcel OB), cellulose *tris*(4-methylbenzoate) (Chiralcel OJ). The obtained results on different columns were compared.

EXPERIMENTAL

The instrument used was a Shimadzu HPLC system with the following specifications; SCL-10A vp system controller, SIL-10AD vp auto injector, two LC-10AT vp pumps, SPD-10AV vp UV/VIS detector, CTO-10AC vp oven, Shimadzu GT-154 degasser, Class vp, version 4.2, chromatography

data system for data acquisition and analysis. The computer used for generation a database was Pentium II, 56.0MB Ram, Microsoft windows 95. Microsoft® Access 97, copyright© 1989-1996 Microsoft cooperation, product ID 63854-335-1671922-74405. Accord for Microsoft Access 3.0, Access 97 Edition, Copyright© 1995-1998 Synopsys Scientific System Ltd., Licence : AC0380-9282.

Chromatographic conditions: The experiments were performed at a constant flow-rate of 1mL/min and a constant temperature of 25ºC. The eluant was detected at 254 nm. The mobile phase consists of HPLC-grade hexane and isopropyl alcohol.

Columns and chemicals: The stainless steel columns (25 cm × 4.6 mm) packed with Chiralcel AD [amylose *tris*(3,5-dimethyl phenyl carbamate) coated on silica gel] Fig.1(1), Chiralcel OD [cellulose *tris*(3,5-dimethyl phenyl carbamate) coated on silica gel] Fig.1(2), Chiralpak AS {amylose *tris*[(S)-1-phenyl ethyl carbamate] coated on silica gel} Fig.1 (3), Chiralcel OB [cellulose tribenzoate] coated on silica gel Fig.1(4), Chiralcel OJ [cellulose *tris*(4-methylbenzoate) coated on silica gel] Fig.1(5) were purchased from Daicel Chemical Industries Ltd. Table-1 showed all racemates separated by different CSPs.

RESULTS AND DISCUSSION

One of the problems confronting analytical chemists arises from the need to keep track of and to make sense of, the vast amount of data that can be generated by a busy laboratory. To this end a computerized database is often seen as a useful tool for solving the data storage problem.

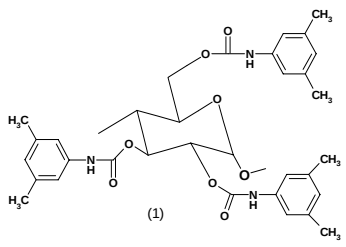
A database may take many forms and its simplicity may be no more than a list of analyses performed by a laboratory in a given week. More data may require a table or may be several tables. This complexity is countered by the relational database. In such a computer program data can be entered and stored in a number of tables. These tables can be linked together in such a way as to allow the relevant data to be displayed from any number of tables. So that, for example, a laboratory manager could interrogate the database to find out which of his analysts had performed a particular analysis, on what days and with what results. This ability to store a lot of data and to access particular aspects of it, has proved to be very useful to analysts working to separate the isomers of compounds exhibiting optical activity *i.e.*, chirality. Currently there is no rational to determine which chiral stationary phases are best suited to separate the enantiomers of a particular chiral compound. Consequently, when a new chiral compound is first synthesized, choice of a suitable chromatographic column and the instrumental operation conditions have to be determined empirically. In attempts to reduce time of method development and materials, databases which contain

details of successful separation of enantiomers have been created, usually by companies for their exclusive use, although there is atleast one in academic circles which allows for access by subscription. The database program must have the ability to handle chemical structure *i.e.*, an awareness of chemistry. It must be able to store chromatograms, analyte structure, text and numeric data and be able to search for specific data even when the data is stored as a chemical structure.

TABLE-1
THE RACEMATES AND THEIR COLUMNS

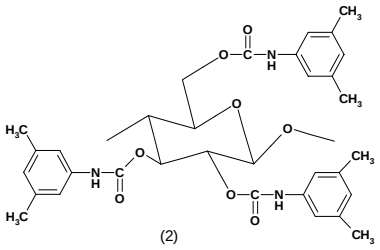
No. of samples	Samples name	CSP
1.	1-(4-Methoxyphenyl)-4-oxo-1,2,3,4-tetrahydropyridine-2-carboxylic acid methyl ester	Chiralpak AD
2.	1-(4-Methoxyphenyl)-4-oxo-1,2,3,4-tetrahydropyridine-2-carboxylic acid ethyl ester	Chiralpak AD
3.	2-Phenylglycinemethyl ester hydrochloride	Chiralpak AD
4.	N-(2-hydroxy-2-phenylethyl)-phthalimide	Chiralpak AD
5.	Methyl 2-hydroxy-2-(1´-naphthyl) acetate	Chiralcel OJ
6.	Ethyl 2-hydroxy-2-(4´-nitrophenyl) acetate	Chiralcel OJ
7.	1-Benzosuberol	Chiralcel OJ
8.	2-Nitro-1-phenylpropane	Chiralcel OJ
9.	1-Naphthyl oxirane	Chiralcel OJ
10.	2,2,2-Trifluoro-1-(9-anthryl) ethanol	Chiralcel OJ
11.	Trans-stibene oxide	Chiralcel OJ
12.	Epinephrine	Chiralcel OB
13.	1-Tetralol	Chiralcel OB
14.	1-Indanol	Chiralcel OB
15.	4-Chromanol	Chiralcel OB
16.	2-Naphthyl oxirane	Chiralcel OB
17.	Ethyl-3-phenyl butyrate	Chiralcel OB
18.	Methyl mandelate	Chiralcel OD
19.	Methyl-2-hydroxy-2-(4´-methoxyphenyl)acetate	Chiralcel OD
20.	Methyl-2-(4´-chlorophenyl)-2-hydroxyacetate	Chiralcel OD
21.	Ethyl-2-hydroxy-4-phenylbutyrate	Chiralcel OD
22.	Methyl-2-hydroxy-4-phenylbutyrate	Chiralcel OD
23.	Ethyl mandelate	Chiralcel OD
24.	9-Anthracyl oxirane	Chiralcel OD
25.	2-Methyl-3-pheny-1-propanol	Chiralcel OD
26.	Ethyl-2-(4´-chlorophenyl)-2-hydroxyacetate	Chiralpak AS
27.	Methyl-2-hydroxy-2-(4´-methylphenyl)acetate	Chiralpak AS
28.	Methyl-2-hydroxy-2-(2´-naphthyl)acetate	Chiralpak AS

Two software packages were readily available as possible tools for the project.



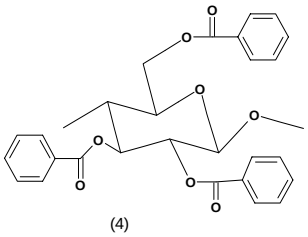
(1)

(Amylose tris (3,5-dimethyl phenyl carbamate) [Chiralpack AD]



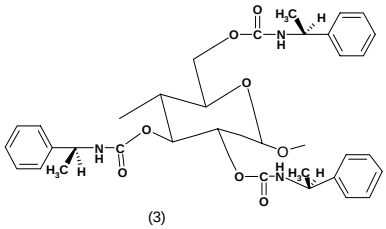
(2)

(Cellulose tris (3,5-dimethyl phenyl carbamate) [Chiralpack OD]



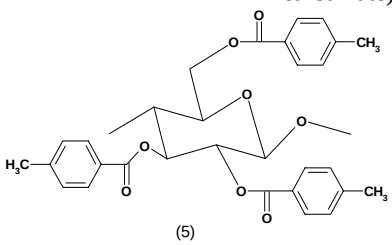
(4)

(Cellulose tribenzoate) [chiralcel OB]



(3)

(Amylose tris [(s)-1-phenyl ethyl carbamate) [Chiralpack AS]



(5)

(Cellulose tris (4-methyl benzoate) [chiracel OJ]

Fig.1 Cellulose and amylose derivatives (CSPs)

Building the program

ISIS base: ISIS base[19] was considered initially as a suitable vehicle for the project because ISIS base and its complementary program ISIS draw, have an awareness of chemistry. ISIS base is able to store analyte structure and search for data, but the shortcoming was its lack of picture handling capability. Therefore it was unable to store chromatograms.

Microsoft Access: Microsoft Access[20] was considered as an alternative, because Microsoft Access is able to store and search data, such as numbers and text, it can even store graphic images. However, when it comes to applying any meaningful operations to chemical moieties, such as calculating molecular weights or searching by structure, Access

provides no in-built mechanisms of handling chemical structures. It is necessary to use a secondary program which can be add to Microsoft Access to give it chemical awareness called accord for Microsoft Access[21].

Accord for Microsoft Access: Accord was developed to enhance the Microsoft access ability to deal with chemical structure composed by ISIS draw and to demonstrate the structure and chromatogram of separation enantiomers in the same time as shown in Fig.2. In this project we are facilitating the separation of enantiomers by constructing database which is able to match the enanatiomer structures and finding out the most suitable column and optimum separation method for the enantiomers.

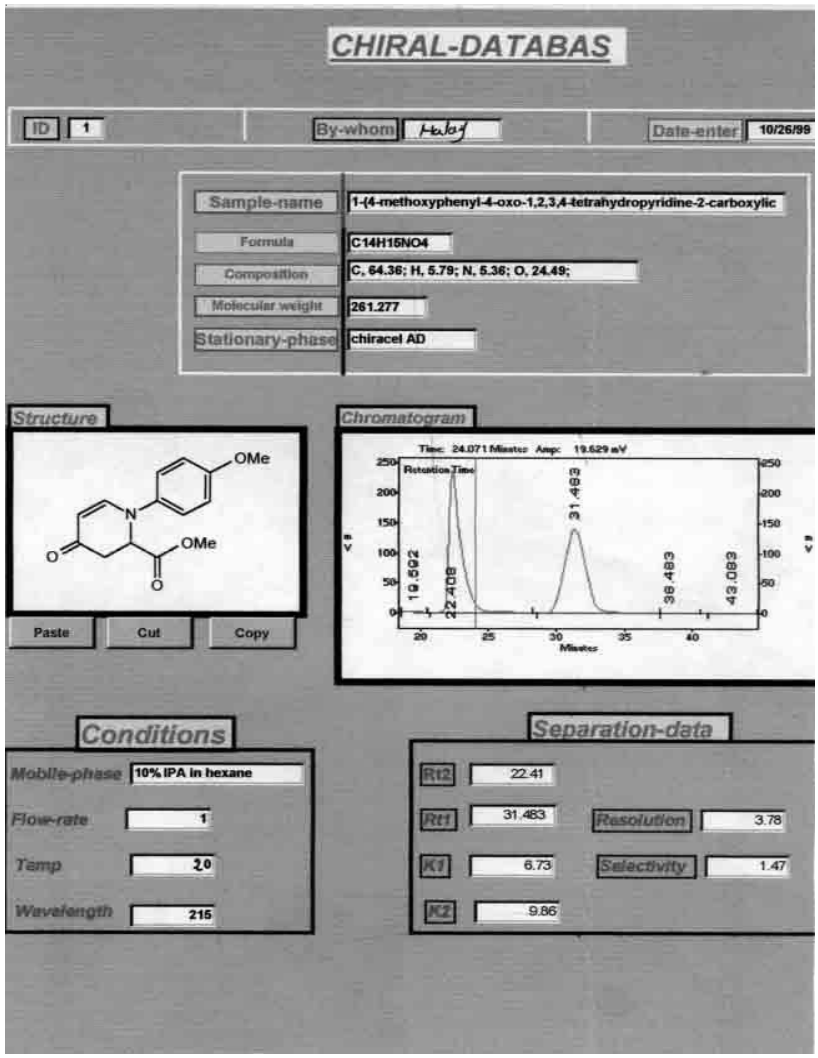


Fig. 2. Form in the final picture for one record

Actual steps: The actual steps for carrying out the project including.

(1) Creating a table which is mainly consists of stationary phase, sample name, chromatogram, structure, mobile phase, flow rate, wave length, R1, R2, resolution and selectivity, (2) A friendly user interface to enable easy use of the program. This interface is able to display chemical properties such as formula, molecular weight or per cent composition and (3) building chemical query tool which is able to match a chemical structure or part of the structure and functional group of the structure of enantiomers.

Feeding the Database: After forming the database it was necessary to input information about the separation of twenty-eight enantiomers using the five chiral stationary phases (CSPs) available in the Chemistry department at UMIST as shown in Fig.2 which represents the out put of one record in this work in order to test the program.

Separation of Compounds on the Chiral Stationary Phases (csps): Cellulose and amylose are the most accessible naturally occurring optically active polymers. These polysaccharide themselves show chiral recognition[22,23], but do not afford practical CSPs. However, their derivatization (phenyl carbamates and esters) brings about practically useful CSPs with high chiral recognition that can separate a wide range of racemic compounds into enantiomers. Among various kinds of cellulose esters, cellulose tribenzoate and cellulose *tris*(4-methylbenzoate) [Fig.1(4) and Fig.1(5)]. The optical resolving abilities of the benzoate derivatives are dependent on the substituents on the phenyl groups. The most important adsorbing site of the derivatives may be the carbonyl group and its polarity may be changed by the substituents on the phenyl groups. The derivatives may interact with racemic compounds having carbonyls groups through dipole-dipole interactions and with compounds having hydroxyl or amino groups through hydrogen bonding. The benzoates having an electron donating substituents such as methyl group showed a high chiral recognition which increases the electron density on the carbonyl oxygen leading to strong interaction with the solutes [Fig.1(3), Fig.1(4)]. Cellulose phenyl carbamates (chiralcel OD) [Fig.1 (2)] shows a particularly high optically resolving ability. Amylose phenyl carbamates (chiralpak AD and chiralpak AS) [Fig. 1(3)] also shows chiral recognition.

The optical resolving abilities of the amylose derivatives are different from those of the corresponding cellulose derivatives and are complementary to each other. The binding of the solutes to the phenyl carbamates cellulose and amylose was achieved through interactions between the solutes and the polar carbamate groups on the CSPs. The carbamate groups on the CSPs can interact with solutes through hydrogen bonding using the (C=O) and NH groups and through dipole-dipole interactions using the C=O moiety[24,25]. Wainer *et al.*[9] have reported that the solute-CSP complex, formed

between a solute having aromatic functionalities and a CSP can be stabilized by insertion of the aromatic portion of the solute into the chiral cavity. Thus the stabilization interaction might also occur due to the presence of the aromatic functionalities on the solutes. Chiral discrimination between the enantiomers may be due to the differences in their steric fit in the chiral cavities.

Comparison of the retention, selectivity and resolution on the CSPs (AD and OD): Amylose derivatives AD showed stronger retention, lower selectivity and lower resolution of the solutes as shown in Table-2 compared to its cellulose based counter part OD column as shown in Table-3. Since the derivatization group on both CSPs (AD and OD) is the same, the difference retention, selectivity and resolution behaviours of these columns should be due to the conformational difference between the amylose and cellulose and also the difference of geometrical structure of the solutes.

TABLE-2
RETENTION TIME, CAPACITY FACTOR, RESOLUTION AND SELECTIVITY OF THE ANALYZED SAMPLES ON CHIRALPAK AD

	Sample(1)	Sample(2)	Sample(3)	Sample(4)
$t_{0\ (mm)}$	2.90	2.90	2.90	2.90
$t_{1\ (mm)}$	22.41	20.90	7.05	15.48
$t_{2\ (mm)}$	31.48	28.30	8.23	17.83
K'_1	6.73	6.21	1.43	4.34
K'_2	9.86	8.76	1.84	5.15
$R_{(s)}$	3.78	4.49	2.49	1.70
α	1.47	1.41	1.28	1.18

TABLE-3
RETENTION TIME, CAPACITY FACTOR, RESOLUTION AND SELECTIVITY OF THE ANALYZED SAMPLES ON CHIRALCEL OD

	Sample (18)	Sample (19)	Sample (20)	Sample (21)	Sample (22)	Sample (23)	Sample (24)	Sample (25)
$t_{0\ (mm)}$	2.90	2.90	2.90	2.90	2.90	2.90	2.90	2.90
$t_{1\ (mm)}$	7.57	9.40	7.45	6.25	8.46	4.77	6.25	16.76
$t_{2\ (mm)}$	12.16	14.39	8.29	8.97	10.74	6.36	7.47	20.62
K'_1	1.61	2.24	1.57	1.16	1.92	0.65	1.16	4.78
K'_2	3.20	3.96	1.86	2.09	2.70	1.20	1.58	6.11
$R_{(s)}$	8.52	3.25	2.13	6.90	3.50	5.84	2.50	5.06
α	1.98	1.76	1.18	1.80	1.40	1.85	1.36	1.28

Comparison of the retention, selectivity, and resolution on the CSPs (OJ and OB): Cellulose *tris*(4-methylbenzoate) (Chiralcel OJ) showed stronger retention, lower selectivity and lower resolution as shown in Table-4 compared to cellulose tribenzoate (Chiralcel OB) as shown in Table-5. It was found that benzoates (chiralcel OJ), have a mild electron donating substituents as a methyl group showed a higher chiral recognition[26] and also the difference of the result may be due to the conformation different between Chiralcel OJ and Chiralcel OB and the difference structure of the solutes.

TABLE-4
RETENTION TIME, CAPACITY FACTOR, RESOLUTION AND SELECTIVITY OF THE ANALYZED SAMPLES ON CHIRALCEL OJ

	Sample (5)	Sample (6)	Sample (7)	Sample (8)	Sample (9)	Sample (10)	Sample (11)
$t_{0\ (mm)}$	2.90	2.90	2.90	2.90	2.90	2.90	2.90
$t_{1\ (mm)}$	27.47	19.46	6.48	22.70	7.75	12.38	6.68
$t_{2\ (mm)}$	31.13	22.79	7.83	24.90	9.60	14.47	7.95
K'_1	8.47	5.71	1.24	6.82	1.67	3.27	1.30
K'_2	9.73	6.86	1.70	7.58	2.31	3.99	1.74
$R_{(s)}$	1.48	2.18	2.58	2.32	2.78	1.18	2.40
α	1.15	1.20	1.37	1.11	1.38	1.22	1.33

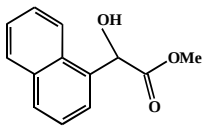
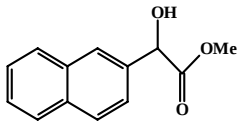
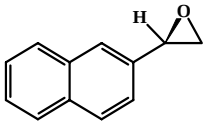
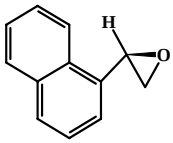
TABLE-5
RETENTION TIME, CAPACITY FACTOR, RESOLUTION AND SELECTIVITY OF THE ANALYZED SAMPLES ON CHIRALCEL OB

	Sample (12)	Sample (13)	Sample (14)	Sample (15)	Sample (16)	Sample (17)
$t_{0\ (mm)}$	2.90	2.90	2.90	2.90	2.90	2.90
$t_{1\ (mm)}$	3.19	4.64	5.05	6.46	8.36	5.00
$t_{2\ (mm)}$	3.68	6.38	6.83	8.09	9.28	5.57
K'_1	0.10	0.62	0.74	1.23	1.88	4.88
K'_2	0.27	1.20	1.35	1.79	2.20	5.55
$R_{(s)}$	1.80	4.11	4.48	3.01	1.41	1.49
α	2.70	1.93	1.35	1.45	1.17	1.14

CSPs for HPLC have usually been prepared with either chiral small molecules or polymers with optical resolving power[26]. The CSPs derived from small molecules are prepared by chemically bonding the chiral small molecules to a support, usually silica gel and their chiral recognition can be predictable from that of the chiral small molecules themselves. On the other

hand, chiral recognition of polymeric CSPs often depends on the higher order structure of the chiral polymer and therefore, it is difficult to predict, their chiral recognition only from the character of a monomer unit. The multiple interaction possibilities provided by the polysaccharide derivatives make prediction of the separation very difficult. However it should be remembered that these multiple interaction sites that provide these phases with their broad applicability and hence the reason why they are so extensively used. The complex nature of these interactions will probably mean that the resolution mechanisms on the polysaccharide derivatives will remain obscure for some time to come. This can be described by some of the following enantiomers as examples which were carried out on the columns in this work. Though these enantiomers are highly similar, they have been separated on different columns as shown in Table-6 that may be attributed to different conformation of CSPs and may depend on the geometrical change of the molecule. So it is difficult to select the suitable CSP for a particular separation. As a result, the database is generated for helping to select the most suitable column for particular separation.

TABLE-6
SEPARATION OF SIMILAR RACEMATES BY DIFFERENT COLUMNS

No.	Sample structure	Sample name	CSP name
5-		Methyl-2-hydroxy-2-(1´-naphthyl) acetate	Chiralcel OJ
28-		Methyl-2-hydroxy-2-(2´-naphthyl) acetate	Chiralpak AS
16-		2-Naphthyl oxirane	Chiralcel OB
9-		1-Naphthyl oxirane	Chiralcel OJ

Database validation and ability: Finally to prove the ability of the database for selection of a suitable column, the structure of methyl mandelate was inserted as a query in the database. By searching, it is found that the compound ethyl mandelate highly similar to methyl mandelate and it

was separated on chiralcel OD as shown in Fig.3. By using the chiralcel OD for separation the enantiomer of methyl mandelate, it gave a successful separation as shown in Fig.4 which indicated the ability of the database for choosing the suitable column for particular separation.

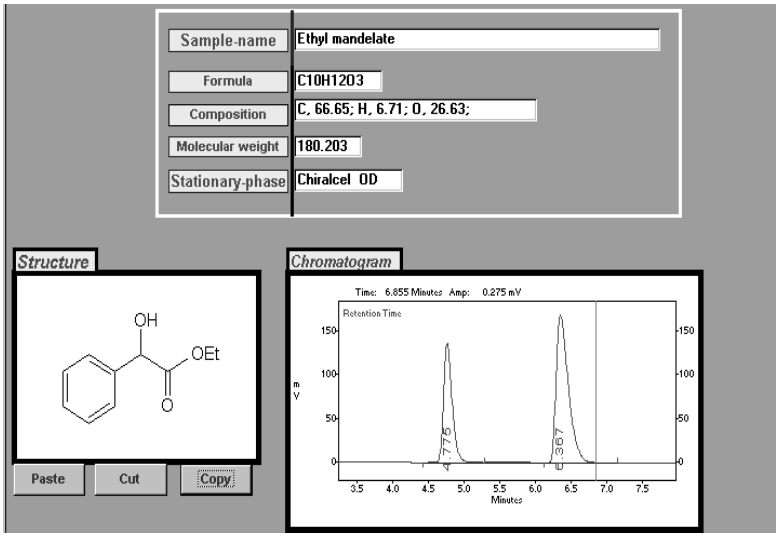


Fig.3 Record of Ethyl mandelate

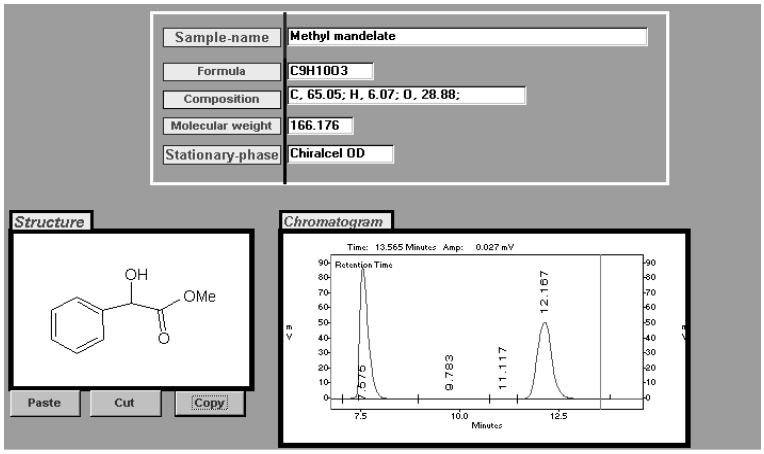


Fig. 4. Record of Methyl mandelate

The data base was successfully validated for academic purposes however going commercial requires richer database which includes many chiral stationary phases and enantiomers structure and their chromatograms. This will enable the program to be applicable in the commercial market.

Conclusion

Achievement of this work:

(1) The Database of chiral HPLC separations was constructed by using software called Accord for Microsoft Access, (2) The chromatogram was inserted in the database which gave a complete picture of the separation, (3) As a testing sample the database was fed by twenty eight enantiomers separated by using five chiral stationary phases (csps), (4) It is recommended to use the program after enhancing its abilities by enriching the data base and (5) Using this developed program can save time, materials and labour for selection the column and the optimum method of separation of racemates.

REFERENCES

1. D. Burk and D. Henderson, *Br. J. Anaesthesia*, **88**, 563 (2002).
2. R.L. Snyder, J. Kirkland and L. Glajch, Practical HPLC method Development, Wiely, New York, edn. 2, p.537 (1997).
3. G.S. Allenmaerk, Chromatographic Enantioseparations methods and application, Ellis Horwood, Chichester, p. 90 (1988).
4. R.D. Talyer and K. Maher, *J. Chromatogr. Sci.*, **30**, 67 (1992).
5. A.V. Davankov, *J. Chromatogr. A*, **666**, 55 (1994).
6. J. Buechler, M. Schwab, G. Mikus, B. Fischer, L. Hermle, C. Marx, G. George, M. Spitzer and K. Arturovar, *J. Chromatogr. B*, **793**, 207 (2003).
7. B. Ilia, M. Dimitris, K. Uwe, E. Dag, H. Erlend, N. Marina, S. Felix, D. Gasan and R. Johan, *J. Chromatogr. B*, **800**, 211 (2004).
8. W. Wainer and C. Alembic, *J. Chromatogr.*, **358**, 85 (1986).
9. W. Wainer, C. Alembic and E. Smith, *J. Chromatogr.*, **388**, 65 (1987).
10. K. Balmer, O. Laggerstorm, A, Person and G. Schill, *J. Chromatogr.*, **592**, 331 (1992).
11. T. O'Brien, L. Croker, K. Thompson, H.P. Toma, A.D. Conlon, B. Feibush, C. Moeder, G. Bicker and N. Grinberg, *Anal. Chem.*, **69**, 1999 (1997).
12. Y. Okamoto, R. Aburatani, Y. Kaida, K. Hatada and K. Chem, *Chem. Lett.*, 1125 (1988).
13. W.H. Pirkle, J.M. Finn, J.L. Schreiner and B.C. Hamper, *J. Am. Chem. Soc.*, **103**, 3964 (1981).
14. T. Shinbo, T. Yamaguchi, K. Nishimura and M. Sugiura, *J. Chromatogr.*, **405**, 145 (1987).
15. W.L. Hinze, T.E. Riehl, D.W. Armstrong, W. Demond, A. Alak and T. Ward, *Anal. Chem.*, **57**, 237 (1985).
16. D.W. Armstrong, T.J. Ward, R.D. Armstrong and T.E. Beesley, *J. Chromatogr. Sci.*, **232**, 1132 (1986).
17. H.Y. Aboul-Enein and M.J. Rafiqual, *J. Liq. Chromatogr.*, **13**, 485 (1990).
18. MDL Information system Inc., ISIS/Base, 14600 Catalina Street, San Leandro, CA 94577 (1998).
19. A. Simposon, Microsoft Access SYBEX Inc. (1993).
20. W.L. Wainer, C.M. Alembic and E. Smith, *J. Chromatogr.*, **388**, 65 (1987).
21. S. Barker and D. Barker, Using Microsoft Access, Que Coperation Indiana, USA (1998).
22. Y. Okamoto, R. Aburatani, Y. Kaida, K. Hatada and K. Chem, *Chem. Lett.*, 1125 (1988).
23. E. Yashima and Y. Okamoto, *Bull. Chem. Soc. (Japan)*, **68**, 3289 (1995).
24. T. Wang and W.Y. Chen, *J. Chromatogr. A*, **855**, 411 (1999).
25. Y. Okamoto, K. Hatano, R. Aburatani and K. Hatadda, *Chem. Lett.*, 715 (1989).
26. M.A. Krstulovic, Chiral Separation by HPLC, Ell is Horwood, Chichester, (1989).

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