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# Some recent high-performance liquid chromatography separations of the enantiomers of pharmaceuticals and other compounds using the Whelk-O 1 chiral stationary phase

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**Abstract**

The Whelk-O 1 chiral stationary phase is generally useful for the chromatographic separation of the enantiomers of many classes of analytes including aryl propionic acid non-steroidal anti-inflammatory drugs, aryl epoxides, sulfoxides, alcohols, amides and esters. We herein report some additional recent enantioseparations obtained with this column, including a number of pharmaceuticals such as thalidomide, nicardipine, isradipine, mephenytoin, nirvanol, cyclandelate, bendroflumethiazide, bupivicaine, tolperisone, proglumide, tropicamide and indapamide. The separation of the enantiomers of a collection of additional analytes is also reported, including several compounds containing basic nitrogen, a heretofore difficult class of compounds to resolve with this column.

*Keywords:* Chiral stationary phases, LC; Enantiomer separation; Pharmaceutical analysis

## 1. Introduction

The Whelk-O 1 chiral stationary phase (CSP) is a valuable column for the high-performance liquid chromatography resolution of the enantiomers of a wide variety of compounds. Originally developed for separating the enantiomers of naproxen [1,2], the column also resolves the enantiomers of most of the related "profens" and was further shown to be useful as a broad spectrum CSP for resolving the enantiomers of the commonly occurring class of compounds possessing a π-aromatic system with a hydrogen bond acceptor located near the stereogenic center (Fig. 1) [3], Subsequently, the Whelk-O 1 CSP has been widely used in the separation of the enantiomers of many compounds fitting this general description [4–16]. We herein report a number of new separations obtained with this column, with special emphasis on compounds of pharmaceutical interest.

## 2. Experimental

### 2.1. Apparatus

Chromatographic analysis was performed using a Kratos ABI Spectroflow 400 pump, a Rheodyne Model 7010 injector fitted with a 10-μl sample loop, a Kratos Spectroflow 783 variable-wavelength ab-

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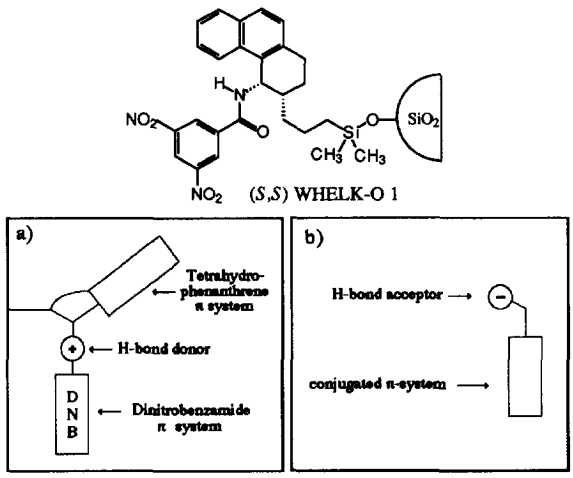


Fig. 1. (a) Schematic diagram showing key functional groups of the Whelk-O 1 CSP involved in chiral recognition. (b) Schematic diagram showing generalized structure of analytes which are resolved on the Whelk-O 1 CSP.

sorbance monitor, and a Hewlett-Packard HP 3396 integrating recorder.

### 2.2. Materials

Analytical (25 cm×4.6 mm I.D. length) (*S,S*) Whelk-O CSP (No. 786101) and (*R,R*) Whelk-O 1 CSP (No. 786201) were obtained from Regis Technologies (Morton Grove, IL, USA). The analytes 1,2,3,4-tetrahydro-2-naphthoic acid, α-trityl-2-naphthalenepropionic acid, 2-phenylcyclopropane carboxylic acid, Troger's base, EEDQ, *trans*-2-phenylcyclohexanol, tetrahydrobenzopyrene-7-ol, tetrahydronaphthol, 2-methyl-1-tetralone, 2-methyl-1-indanone, 3-methyl-1-indanone, 3a,4,5,6-tetrahydrosuccinimido[3,4-*b*]acenaphthen-10-one, methyl *trans*-3-(4-methoxyphenyl)glycidate, phenylsuccinic anhydride, mandelic acid and methyl mandelate were obtained from Aldrich (Milwaukee, WI, USA). The analytes thalidomide, bendroflumethiazide, bupivicaine, tolperisone, cyclandelate, proglumide, tropicamide, and indapamide were obtained from Sigma (St. Louis, MO, USA). The analytes isradipine and nicardipine were obtained from RBI, Natick, MA, USA. The hydroxyphosphonate analytes **7** and **8** were provided by Professor Chris Spilling (University of Missouri, St. Louis, MO, USA). The indole analytes **30** and **31** were provided by Professor James Cook and Linda Hamaker (University of Wisconsin, Milwaukee, WI, USA). The chromatograms of nirvanol and mephytoin were provided by Professor Allan Rettie at the University of Washington.

### 2.3. Methods

Chromatographic analysis was carried out at a flow-rate of 1.0 ml/min. Detection was by UV at either 254 nm or 220 nm.

## 3. Results and discussion

The Whelk-O 1 column, originally developed for the resolution of naproxen enantiomers, continues to prove useful for resolution of the enantiomers of a variety of analytes. Some recent examples are shown in Fig. 2. Several of these compounds, for example, compounds **1** and **2**, bear some structural similarity to the family of non-steroidal anti-inflammatory drugs, which perhaps accounts for their resolution.

Typically a mobile phase containing a small amount of acetic acid is used in the separation of carboxylic acid enantiomers with this CSP, although salts such as ammonium acetate have also been used for this purpose [1,2]. The use of trifluoroacetic acid as a mobile phase additive is discouraged, as this may lead to strand cleavage and irreversible changes to the CSP.

Analyte **3** also fits the general model of an analyte which can be resolved on the Whelk-O phase, although with this compound either the naphthyl or the more sterically bulky trityl group could be used for the π–π interaction required for chiral recognition. This type of competitive situation typically leads to reductions in enantioselectivity [17], although in the present case good resolution is still obtained. In another example, either the epoxide oxygen or the ester functionality of analyte **4** could function as hydrogen bond acceptor in the chiral recognition model, again giving rise to some degree of competition and presumably some decrease in enantioselectivity. To further complicate matters, this analyte also contains an additional methoxyl which can also participate in hydrogen bonding, but not in a sense which increases enantioselectivity, another

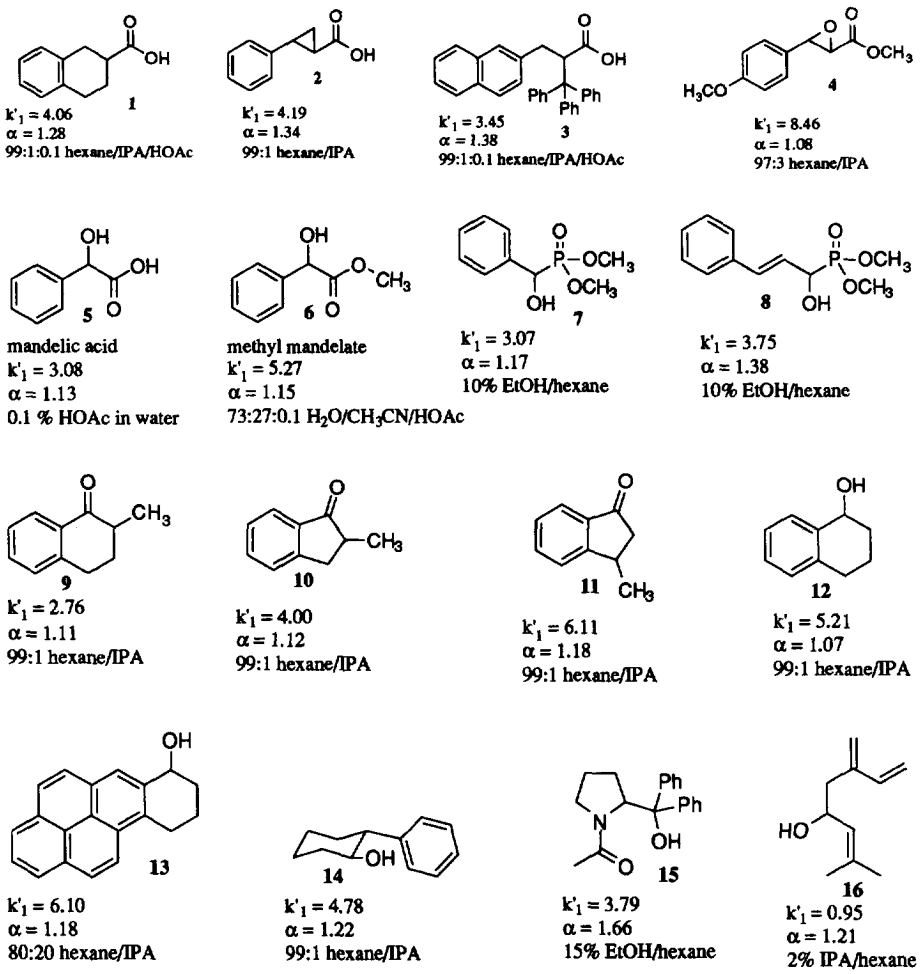


Fig. 2. Separation of the enantiomers of a variety of analytes on the Whelk-O 1 CSP.

factor which usually decreases enantioselectivity [17].

Separation of the enantiomers of the highly hydrophilic analyte, mandelic acid, **5**, was accomplished using a mobile phase of pure water containing 0.1% acetic acid. Such reversed-phase separations using Pirkle CSPs have been known for some time [18], but the vast majority of work done with these columns has employed the use of normal-phase eluents. Recent results such as the separation of the enantiomers of **5** and the corresponding methyl ester, **6**, suggest that the Whelk-O CSP could be more widely useful in the reversed-phase mode than heretofore realized.

Many carboxylic acid derived functionalities can serve as hydrogen bond acceptors, for example, esters, amides, ureas, carbamates [3]. Not surprisingly, the phosphonate functionality can also play this role. A large number of aminophosphonates have previously been resolved on this CSP [3,19]. Here we present the first cases of the resolution of the enantiomers of hydroxyphosphonate **7** and **8** using the Whelk-O column. We have previously reported the separation of enantiomers having phosphorous as the stereogenic center using the Whelk-O CSP [3].

Cyclic ketones **9–11** and the corresponding alcohols such as **12** and **13** are also resolved on this column. Alcohols related to **14** have proven useful as chiral auxiliaries [20]. This compound, the corresponding ketone and a number of analogs are all well

resolved on the Whelk-O CSP [3]. The N-acylated prolinol, **15**, is derived from the ligand which is widely used in enantioselective borane reductions [21]. The excellent resolution provided in this case suggests that the Whelk-O 1 column may be useful for resolving analogs of this ligand.

The separation of the enantiomers of the terpene natural product, ipsdienol, **16**, is an interesting, but by no means unique example of the resolution of enantiomers lacking an aromatic ring. The compound does have a conjugated π-system which is presumably participating in the chiral recognition process. Several separations of enantiomers which have no aromatic groups have been performed with this column, including the sesquiterpene plant growth regulator abscisic acid [5].

Hydantoin compounds have been resolved on π-acidic Pirkle columns for many years, and hydantoin-based CSPs were even developed by Pirkle and Hyun [22]. It is therefore not surprising that drugs such as the anticonvulsants, mephenytoin, **17**, and nirvanol, **18**, are resolved on the most recent π-acidic Pirkle column, the Whelk-O 1 CSP. The quality of these separations is nevertheless impressive, and suggests that other members of the hydantoin and related barbiturate families may also be resolved.

As discussed above, many compounds possess, in addition to those interaction sites called for in the chiral recognition model, superfluous sites which tend to complicate matters and generally make resolutions difficult or problematic [17]. An example is provided by the infamous sedative/teratogen, thalidomide, **19**. The structure of thalidomide is peppered with a variety of strongly polar functionality in addition to that which is required for chiral recognition by the Whelk-O phase. Nevertheless, an acceptable separation is obtained in the reversed-phase mode with addition of a small amount of acetic acid to improve band shape. The related imide, **20**, also contains an abundance of strongly interacting functionality, however the compound is conformationally rigid and has a geometric distribution of interaction sites which is more favorable for interaction with the CSP. Nevertheless, the analogous compound in which all but the essential interaction sites were removed would doubtlessly perform better.

The antihypertensive diuretics containing a sulfonamide moiety are a very polar class of compounds which can sometimes be difficult to resolve. The strong retention afforded by the sulfonamide moiety can to some degree be overcome by employing a mobile phase with more polar modifier. In these examples a higher concentration of isopropyl alcohol (IPA) has been used. Other options would include the use of ternary mobile phases containing additives such as dichloromethane or acetonitrile in addition to IPA–hexane.

Another class of antihypertensives which is the subject of much recent study is the dihydropyridine calcium channel blockers. Interestingly, these compounds are chiral only by virtue of their differentially substituted carboxylic acid groups. For isradipine, **23**, this corresponds to the difference between a methyl and an isopropyl ester. While this is by no means a large difference, the enantiomers can nevertheless be resolved using a reversed-phase eluent, as shown in Fig. 3, or using a more typical normal-phase eluent of 5% IPA in hexane with 0.5% triethylamine (not shown). The difference between the two ester substituents of nicardipine, **24**, is much greater, although the presence of a basic amino group in the molecule complicates matters somewhat.

Historically, basic amino compounds have not been well resolved on Pirkle CSPs owing to the very strong retention of these compounds on CSPs containing electron deficient dinitrobenzoyl aromatic rings. Recently we have found that these problems can be overcome, in full or in part, by the judicious use of mobile phase additives. For example, addition of a small amount of triethylamine to the eluent permits good resolution of the enantiomers of the local anesthetic, bupivicaine, **25**, the muscle relaxant, tolperisone, **26**, or the anticholinergic, tropicamide, **27**. In the case of the anticholinergic, proglumide, **28**, the best separation was afforded when acetic acid was used as a mobile phase modifier. A variety of amines and carboxylic acids have been used as polar modifiers with this CSP, and those shown here are intended only to provide representative examples and are by no means fully optimized.

In the case of Troger's base, which is not a pharmaceutical, but a much studied chiral solvating agent which was first resolved chromatographically

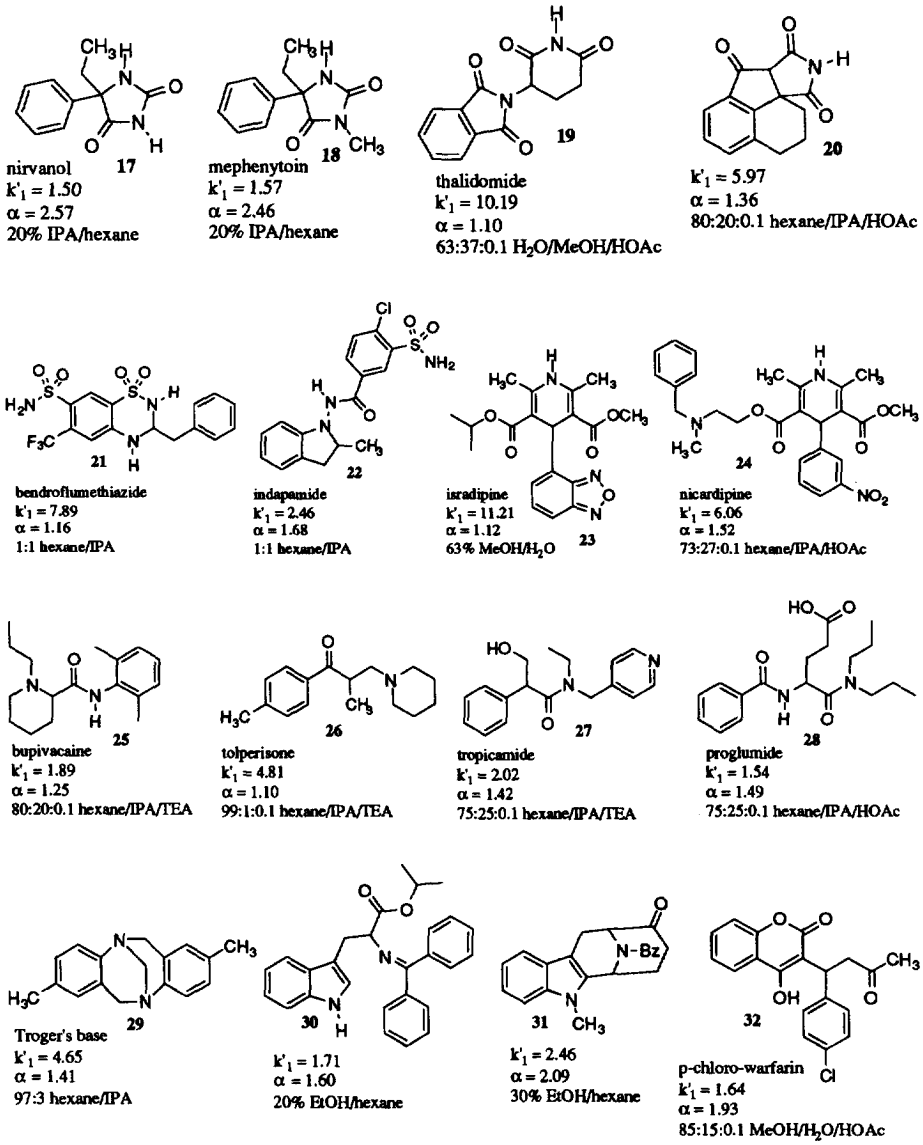


Fig. 3. Separation of the enantiomers of a variety of pharmaceutical analytes on the Whelk-O 1 CSP.

more than 50 years ago [23], a simple IPA–hexane mobile phase is sufficient for resolution without addition of any extra polar modifiers. Similarly the imino indole, **30**, and the related compound, **31**, require only a mobile phase of ethanol in hexane. In general, highly substituted or sterically bulky amines present less of a problem with this CSP, and often times no special additives may be needed in their enantioseparation.

Separation of the enantiomers of the anticoagulant, warfarin, using the Whelk-O CSP was reported earlier [3]. The preparative separation of warfarin enantiomers on this CSP using supercritical $CO_2$ eluents has also been described [7]. Warfarin enantiomers can also be resolved on this CSP under reversed-phase conditions (methanol–water–acetic acid, 85:15.0.1; $k'_1 = 1.64$; $\alpha = 1.93$). In addition, the separation of the enantiomers of the *p*-chloro analog

of warfarin, **32**, are shown. It is generally the case, especially when dealing with a compound whose enantiomers are well separated, that the column will also be able to resolve a large number of structural analogs. This is a very valuable property, especially useful for examining combinatorial libraries or other large collections of closely related compounds.

## 4. Summary and conclusion

The separations of the enantiomers of a number of pharmaceutical and non-pharmaceutical analytes with the Whelk-O CSP has been described. A selection of analytes from new compound classes and from pharmaceutical classes of some interest have been chosen to emphasize the generality of this CSP. It is hoped that the presentation of these examples will give users a sense of the type of analytes which are likely to be resolved with this column and some idea of what type of conditions should be used.

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