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Twenty Years of Separation of Cis-Trans (Z)-(E) Isomers

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Twenty Years of Separation of Cis-Trans (Z)-(E) Isomers

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Abstract: This review presents a concise survey of articles dealing with the separation of cis-trans (Z)-(E) isomers. The geometric isomerism generated by ethylene bonds (carbon-carbon double bond) is the only one treated. An introduction focuses on the existing chemical nomenclature and the encountered problems in modern analytical and pharmaceutical chemistry. Next, the study is divided into three parts: synthetic compounds, natural compounds, and fatty acids and vitamins. Within this framework, articles have been selected to describe past and current trends in analytical chemistry and outline relevant research interests. Afterwards, the different mechanistic approaches that could explain the separation of such geometrical isomers is reviewed. These approaches may be used to design and to describe more interesting methodologies.

Keywords: Separation, Z-E isomers, HPLC, SFC, capillary electrophoresis

INTRODUCTION

Compounds that have the same molecular formula but different chemical structures are called *isomers*. Owing to the differences between the structures, it is possible to classify these into various subtypes, which are described below. For each of these, some examples will be given at the end of this

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introduction (Table 1). Definitions are given in accordance with the IUPAC Compendium of Chemical Terminology (1).

Constitutional isomerism designates the relationship between structures differing in *constitution* and described by different line formulae (for example CH_3OCH_3 and CH_3CH_2OH). Isomerism due to differences in the spatial arrangement of atoms without any difference in connectivity or bond multiplicity between the isomers is called *stereoisomerism*. The stereoisomers can further be divided into *conformational isomers* also named *conformers* and *configurational isomers*.

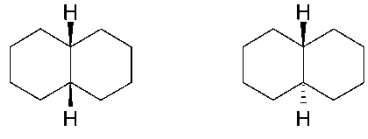
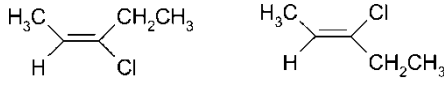
Configurational isomers are in turn shared out amongst *geometric isomers* (an obsolete synonym for cis-trans *isomerism*) and *optical isomers* (i.e., stereoisomers with different optical activities; they should be described as *diastereoisomers* or *enantiomers*). An *enantiomer* can be defined as one of a pair of molecular entities which are mirror images of each other and non-superimposable. *Diastereoisomers* (or *diastereomers*) are stereoisomers not related as mirror images; they are characterized by differences in physical properties, and by some differences in chemical behavior towards achiral as well as chiral reagents.

Cis-trans *isomers* are stereoisomeric olefins or cycloalkanes (or heteroanalogues), which differ in the position of atoms (or groups) relative to a carbon axis; in the cis-isomer the corresponding atoms or groups are on the same side, whereas in the trans-isomer they are on opposite sides.

The terms *E* (from the German "entgegen": opposite) and *Z* (from the German "zusammen," or together) are the approved stereodescriptors of stereoisomeric alkenes, cumulenes and relative systems, when four different subtituents are attached to the sp^2-hybridized carbons of the double bond. The group of highest CIP priority (rules described by Cahn et al. (2)) attached to one of the terminal doubly bonded atoms of the alkene or cumulene is compared with the group of highest precedence attached to the other. The stereoisomer is designated as *Z* if the groups lie on the same side of a reference plane passing through the double bond and perpendicular to the plane containing the bonds linking the groups to the double-bonded atoms; the other stereoisomer is designated as *E*. These descriptors may be applied to structures with a fractional bond order between one and two, and to double bonds involving elements other than carbon. They are not used to describe ring substitution relationships. A special nomenclature was developed for the description of isomeric oximes and nitroso compounds. The terms "syn" and "anti" replace "cis" and "trans," respectively.

The separation and quantification of Z-E isomers is an important objective for the analyst, especially when considering pharmaceutical compounds. Authorities such as the European Pharmacopoeia require the control of Z and E isomer contents in drug substances where this type of isomerism can exist. This is consistent with the fact that these isomers can have different biological/pharmacological/toxicological properties (for example, the Z-isomer of tamoxifen is antioestrogenic, whereas the E-isomer

Table 1. Examples of different kinds of isomerism within organic compounds

Isomerism subtype	Examples
Conformational isomerism	 Staggered and eclipsed form of butane
Optical isomerism: enantiomers	 (S)- (left) and (R)- (right) form of 2-chlorobutane
Optical isomerism: diastereoisomers	 (S,R)- (left, starting from above) and (R,R)- (right) 2-bromo-3-chlorobutane
Cis-trans isomerism	 Cis- (left) and trans- (right) isomers of an olefin
	 Cis- (left) and trans- (right) isomers of a cycloalkane
Cis-trans isomerism (continued)	 Cis- (left) and trans- (right) isomers of a decahydronaphthalen (naphthalane, naphthane)
Z-E isomerism	 (E)- (left) and (Z)- (right) isomers of an alkene (3-chloropent-2-ene)

is a full oestrogen agonist) and highlights the need for suitable and reliable separation techniques in this field. Within this context, the FDA's statement for the development of new stereoisomeric drugs insists on the fact that "geometric isomers (and diastereoisomers) therefore should, with the rare exception of cases where in vivo interconversion occurs, be treated as separate drugs and developed accordingly (3). There is no reason to consider developing mixtures of geometric isomers or diastereoisomers unless they fortuitously represent a reasonable fixed dose combination."

In the following four paragraphs, only the cis-trans isomerism generated by an ethylenic bond (carbon-carbon double bond) will be studied. The synthetic compounds will be treated first, followed by the natural compounds. Fatty acids and vitamins are also natural compounds that will be treated last. Other kinds of geometric isomerism (for example those linked with various ring substitutions) will not be treated here. Table 1 lists the different families of isomerisms. Table 2 presents the structure of the two isomers of most of the compounds mentioned in the text.

SYNTHETIC COMPOUNDS

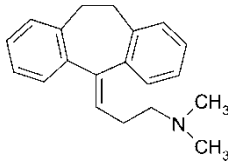
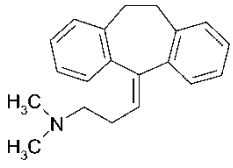
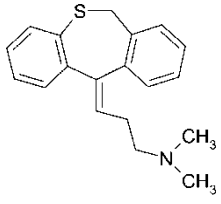
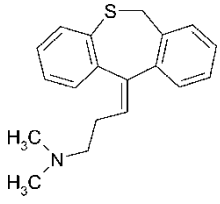
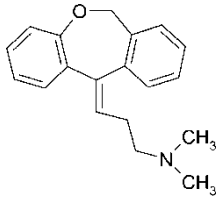
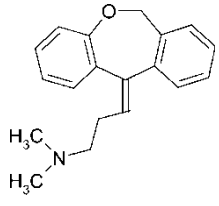
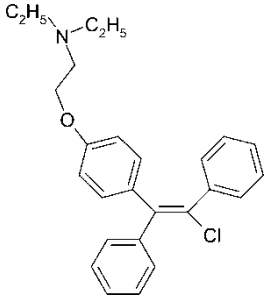
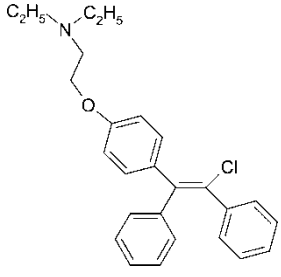
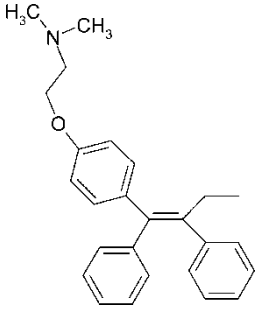
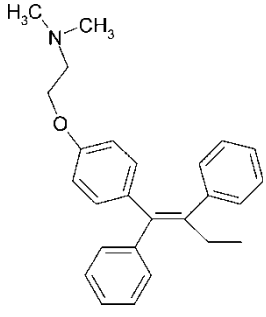
The coverage of this first class of compounds will be divided into 4 main areas: tricyclic antidepressants (imipramine-like antidepressants), estrogen antagonists and agonists-antagonists, thioxanthene derivatives used as neuroleptics and other compounds of interest.

Antidepressants: Imipramine-like Derivatives (Tricyclic Antidepressants)

Amitriptyline [CASRN: 72-69-5] (3-(10,11-dihydro-5H-dibenzo [a,d] cyclohepten-5-ylidene)-N-methyl-1-propanamine) belongs to the imipramine-like derivatives. An HPLC method with a silica column, an aqueous mobile phase containing ammonia and UV detection (254 nm) for the assay of amitryptiline and its main metabolites (amitriptyline-N-oxide, nortriptyline, desmethylnortriptyline, E- and Z-isomers of 10-hydroxyamitriptyline, E- and Z-isomers of 10-hydroxynortriptyline) in the plasma and brain of animals has been achieved (4).

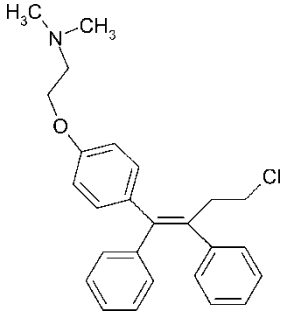
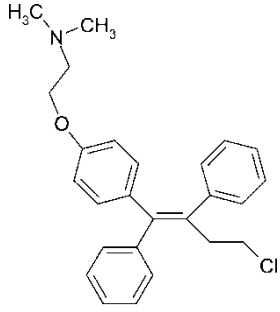
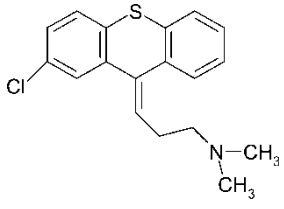
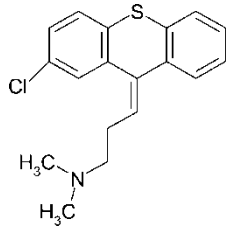
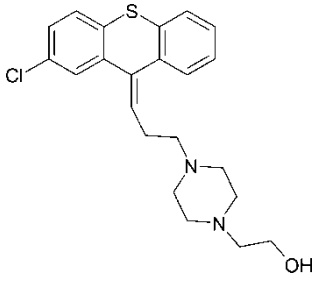
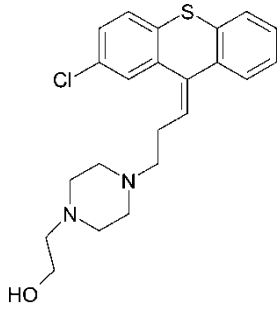
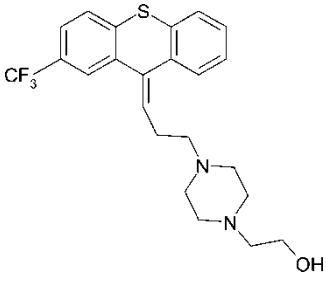
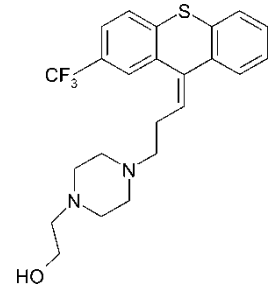
Dosulepin [CASRN: 113-53-1] (3-dibenzo[b,e]thiepin-11(6H)-ylidene-N,N-dimethyl-1-propanamine), and *doxepin* [CASRN: 1668-19-5] (3-dibenz [b,e]oxepin-11-(6H)-ylidene-N,N-dimethyl-1-propanamine), which are respectively dibenzo [b,e] thiepin and dibenz[b,e]oxepin derivatives, also belong to this class of compounds. The (E)- and (Z)-isomers of *dosulepin hydrochloride* [CASRN: 897-15-4] were determined by Pawlak et al. (5) using a HPLC method on a porous graphite carbon column with UV detection (260 nm). Both cis-and trans-isomers of doxepin and desmethyldoxepin were investigated

Table 2. Chemical structure of (Z)- and (E)-isomers of selected compounds (sorted by order of appearance in the text)

DCI	Chemical structure (E)	Chemical structure (Z)
Amitry-ptiline		
Dosulepin		
Doxepin		
Clomiphene		
Tamoxifen		

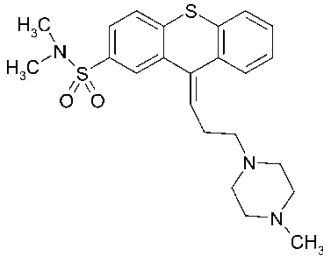
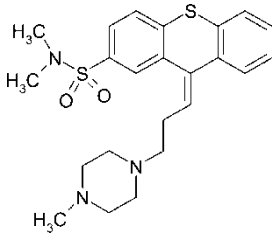
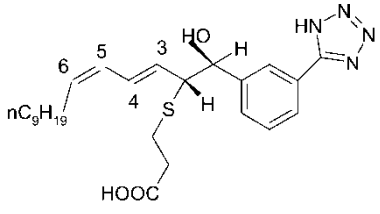
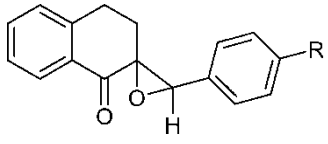
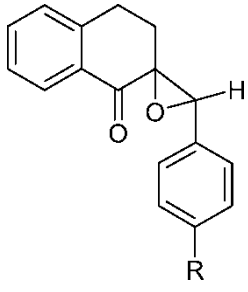
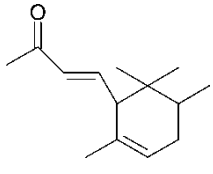
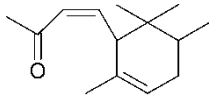
(*continued*)

Table 2. Continued

DCI	Chemical structure (E)	Chemical structure (Z)
Toremifen		
Chlorprothixene		
Clopenthixol		
Flupentixol		

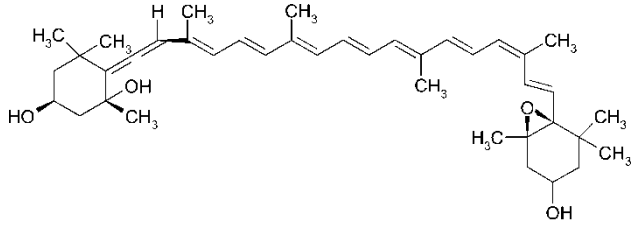
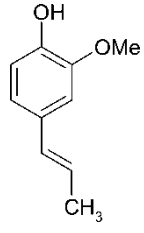
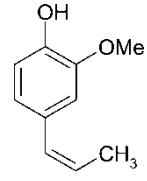
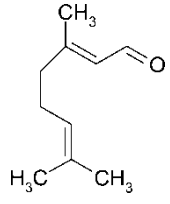
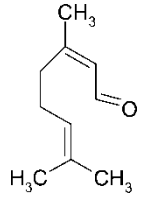
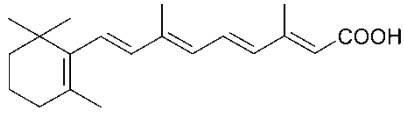
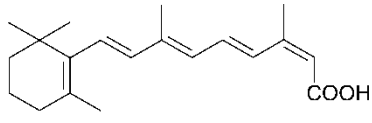
(*continued*)

Table 2. Continued

DCI	Chemical structure (E)	Chemical structure (Z)
Thiothixene		
LY 170680 (Eli Lilly & Co.)	 The figure shows only one (3E, 5Z) of the potential four isomeric arrangements of the conjugated diene system	
2-aryl-methyli-dene-1-tetralones		
α-irone		

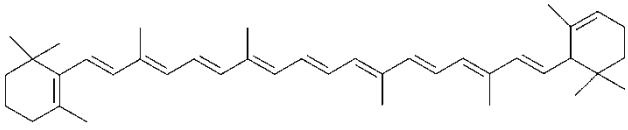
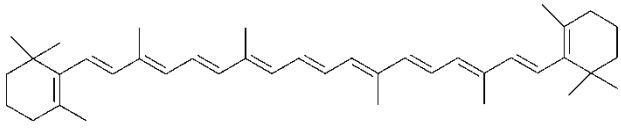
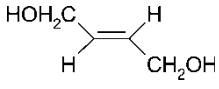
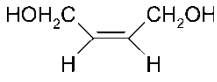
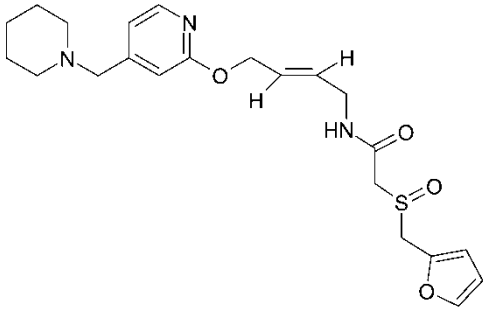
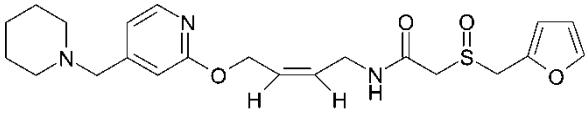
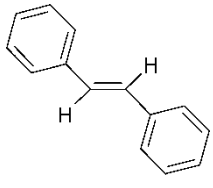
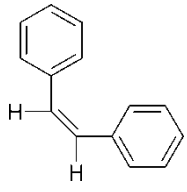
(*continued*)

Table 2. Continued

DCI	Chemical structure (E)	Chemical structure (Z)
Neoxanthin		
	9′Z-(3S, 5R, 6R, 3′S, 5′R, 6′S)-neoxanthin	
Isoeugenol		
Citral		
Retinoic acid		
	all trans (tretinoin)	
		
	13-cis (isotretinoin)	

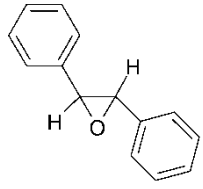
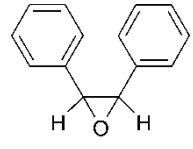
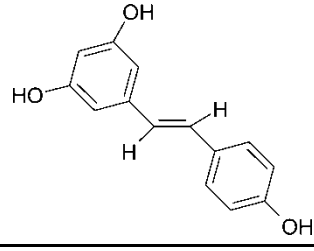
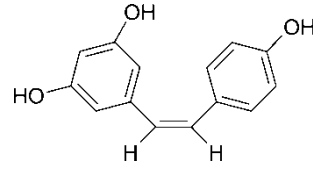
(*continued*)

Table 2. Continued

DCI	Chemical structure (E)	Chemical structure (Z)
Carotene	 all-trans-α-carotene  all-trans-β-carotene	
2-butene-1,4-diol		
Lafutidine	 (E)	
Lafutidine	 (Z)	
Stilbene		

(*continued*)

Table 2. Continued

DCI	Chemical structure (E)	Chemical structure (Z)
Stilbene oxide	 trans	 cis
Resveratrol		

by Hrdina et al. (6) using derivation of the secondary amine and gas chromatography on a capillary column. A normal-phase HPLC procedure with a silica column and a UV detector (254 nm) was described for the simultaneous quantitation of the geometric isomers of both doxepin and N-desmethyldoxepin in plasma and urine (7). Another HPLC approach was furnished by Sokoliess et al. (8), whose team developed a separation of the 2 doxepin isomers on various calixarene- and resorcinarene-bonded HPLC stationary phases, using two different organic modifiers (methanol and acetonitrile) on each column tested.

Three different capillary electrophoretic techniques for the separation of doxepin isomers have been developed. Hu et al. (9) developed two different methods, the first one using a NaH_2PO_4 buffer containing hydroxypropyl-β-cyclodextrin and detection at 297 nm, the second one based on nonaqueous capillary electrophoresis (methanol–acetonitrile–ammonium acetate/glacial acetic acid buffer) with a detection wavelength set at 254 nm (10). Sokoliess et al. (11) studied the separation of these isomers in nonaqueous capillary electrophoresis with calixarenes and resorcinarenes as additives.

Estrogen Antagonists and Agonist-Antagonists

Clomiphene [CASRN: 911-45-5] (2-[4-(2-Chloro-1,2-diphenyl ethenyl) phenoxy]-N,N-diethylethanamine) is a synthetic non-steroidal compound which has both estrogenic and antiestrogenic effects (estrogen agonist-antagonist). It is primarily used for the treatment of anovulatory infertility.

Clomiphene citrate [CASRN: 50-41-9] is a mixture of the *Z* isomer (called zuclomiphene) and the *E* isomer (called enclomiphene).

An HPLC determination of these isomers and their metabolites in human plasma was achieved by Baustian et al. (12) using reversed-phase HPLC (Supelco LC 8 octyldimethylsilyl column with a slightly acid mobile phase and trace amount of silanol screening agent (methanol/water 80:20 v/v with 2.30 mL/L phosphoric acid and 10 μL/L triethylamine). An in-line post-column derivatization reaction (stilbene- to- phenanthrene oxidation) enabled to use fluorescence detection (excitation: 255 nm; emission: 378 nm). Single-dose pharmacokinetics in healthy volunteers of these compounds was also described the same year (13). Another reversed-phase HPLC method (LiChrospher 100 RP-18, mobile phase composition: acetonitrile/methanol/water/ammonium chloride 1%/potassium carbonate 1% 950:30:20:4:8 v/v), also requiring post-column photochemical derivation and subsequent fluorescence detection (excitation: 247 nm; emission: 378 nm), has been developed (14).

Other approaches include capillary electrophoretic separation techniques. Juvancz et al. (15) developed a capillary electrophoretic separation of clomiphene isomers using various derivatives of cyclodextrins (CD) (trimethylated β-CD [TRIMEB], dimethylated β-CD [DIMEB], randomly methylated β-CD [RAMEB], hydroxypropylated β-CD [HPBCD], carboxymethylated β-CD [CMBCD], mixtures of various CD) as additives and investigated the effect of several parameters (background electrolytes, pH and type and concentration of CD). DIMEB was found to provide the most effective separation of clomiphene isomers, yielding a Rs value of more than 14.1 at pH 7; RAMEB, β-CD and HPBCD exhibit similar selectivity profiles to DIMEB, but to a lesser extent. In the case of DIMEB, RAMEB and β-CD, the solubilizing properties of the CDs are used in the separations, whereas the selective complexation features of DIMEB determine the migration of clomiphene in these instances. Mixtures of CMBCD and DIMEB at pH 9 enabled a very fast separation (less than 3 min). Bempong and Honigberg (16) used a Plackett-Burman experimental design (investigating simultaneously buffer ionic strength and pH, cyclodextrin concentration, methanol concentration and injection time) in order to gain information on the optimal set of conditions to use for the separation of clomiphene isomers, also based on a capillary electrophoretic system and cyclodextrin use, whereas Hansen et al. (17) demonstrated the feasibility of this separation without the addition of cyclodextrins, using NACE (non-aqueous capillary electrophoresis).

Tamoxifen [CASRN: 10540-29-1] ((Z)-2-[4-(1,2-diphenyl-1-butenyl) phenoxy]-N,N-dimethylethanamine), a triphenylethylene derivative, belongs to a class of compounds known as steroidal estrogen antagonists (antiestrogen). It is used as a hormonal antineoplasic agent for the treatment of breast cancer. It has to be mentioned that the *Z*-isomer of tamoxifen is antiestrogenic, whereas the *E*-isomer is a full estrogen agonist. Reversed-phase ion-pair chromatography has been successfully performed on tamoxifen (18, 19).

Husain et al. (19) also showed the effect of light on the stability of Z-tamoxifen and its conversion to E-isomer, and applied their chromatographic method to clomiphene using similar conditions. Manns et al. (20) used a statistical technique, which once applied to the solvent selectivity triangle (originally developed by L.R. Snyder (21)), enabled the team to determine the optimal mobile phase composition that had to be prepared for a reversed-phase HPLC analysis of tamoxifen in serum. The detection system utilized post-column ultraviolet irradiation to convert the solutes into their respective photocyclization products, followed by fluorescence detection (excitation: 254 nm; emission: 360 nm).

Toremifen [CASRN: 89778-26-7] (2-[4-[(1Z)-4-chloro-1,2-diphenyl-1-butenyl]phenoxy]-N,N-dimethylethanamine) is also a nonsteroidal antiestrogen, structurally similar to tamoxifen. It is used in the therapy of advanced breast cancer. In 1998, Zhu et al. (22) described a simple method using capillary electrophoresis to separate the Z and E isomers of toremifen.

Neuroleptics: Thioxanthene Derivatives

Chlorprothixene [CASRN: 113-59-7] ((Z)-3-(2-chloro-9H-thioxanthen-9-ylidene)-N,N-dimethyl-1-propanamine) is clinically used as an antipsychotic agent. Hansen et al. (17) succeeded in separating (E)- and (Z)-isomers of chlorprothixene without the addition of cyclodextrins or any surfactants, using NACE (see also under clopenthixol, flupenthixol, thioxanthene and later in this paper). The separation of various thioxanthene derivatives, including chlorprothixene, using HPLC or capillary electrophoresis associated with calixarenes or resorcinarenes has been thoroughly studied by Sokoliess et al. (8, 11, 23).

Clopenthixol [CASRN: 982-24-1] (4-[3-(2-chloro-9H-thioxanthen-9-ylidene)propyl]-1-piperazinethanol) and *flupentixol* [CASRN: 2709-56-0] (4-[3-[2-(Trifluoromethyl)-9H-thioxanthen-9-ylidene]propyl]-1-piperazineethanol) are two other related thioxanthene neuroleptics. The α-flupentixol (Z) is known to show greater pharmacological activity than the β-(E) isomer.

Zuclopenthixol ((Z)-isomer of clopenthixol, also called α-clopenthixol) and its main N-dealkylated metabolite in biological fluids (urine/plasma) have been separated from (E)-clopenthixol as a result of reversed-phase ion-pairing HPLC, with a fluorescence detection (excitation: 260 nm; emission: 435 nm) following an online post-column derivatization of these compounds to thioxanthones (17, 24). (Z)-(E) isomers of clopenthixol, flupenthixol as well as those of flupenthixol decanoate (ester of flupenthixol, thus becoming a long acting injectable antipsychotic compound) have been separated using NACE in acidic media (17). An isocratic, normal-phase HPLC method with a Hypersil cyanopropyl silica column and UV detection at 254 nm for the simultaneous determination of flupentixol geometric isomers and haloperidol (a butyrophenone-derived neuroleptic) in human serum has also been described (25). The separation of various thioxanthene

derivatives, including clopenthixol, using HPLC or capillary electrophoresis associated with calixarenes or resorcinarenes has been, as previously mentioned, thoroughly investigated by Sokoliess et al. (8, 11, 23).

Thiothixene [CASRN: 5591-45-7] (N,N-dimethyl-9-[3-(4-methyl-1-piperazinyl) propylidene] thioxanthene-2-sulfonamide), another thioxanthene derivative, is used as an antipsychotic agent. It is important to remark that the (Z)-isomer of thiothixene shows a greater pharmacological activity than the (E)-isomer.

Severin et al. (26) described an HPLC method suitable for the quantitation and separation of both Z and E-isomers of thiothixene as well as synthetic precursors and degradants in bulk, dosage forms and dissolution samples using a SiAl (silica-alumina) column and an aqueous buffered mobile phase. Another HPLC methodology, using perazine as an internal standard, a Hypersil column and UV detection (230 nm), was proposed by Dilger et al. (27). More recently, Hansen et al. (17) achieved this separation without the addition of cyclodextrins, using NACE.

Other Methods and Synthetic Compounds of Interest

Ion mobility spectrometry/mass spectrometry (IMS/MS) techniques were successfully used by Karpas et al. (28) to derive structural information and differentiate between large isomeric ions up to 55 atoms. Three types of isomers were investigated, including E/Z geometric isomers. Fish (29) described the use of a porous graphitic carbon (PGC) column to separate the four cis and trans isomers of a potential antiasthma agent, LY 170680, and compared the selectivity of porous graphite carbon towards these isomers against that of an ODS-bonded silica. PGC is found to separate the isomers more quickly and to yield much better resolution than ODS-silica does.

Adam et al. (30) achieved the synthesis and separation (by silica gel chromatography) of the isomeric epoxides of 2-arylmethylidene-1-tetralones. The characterization of these products (structure, relative configuration and stereo-chemistry) were elucidated by NMR spectroscopy. Zawisza et al. (31) described the recognition of cis and trans isomers of various azobenzene and azo-crown ethers using voltametric reduction of the adsorbed species. The synthesis, Z/E isomerization (by UV irradiation) and separation of poly-para-phenylenevinylene model compounds were studied (32).

NATURAL COMPOUNDS

Irones

Irones are the pleasantly smelling terpenoids of the orris oil, important in perfume industry, which is extracted from the rhizomes of certain sword-lily of *Iris* species (*I. pallida* var. *dalmatica*, *I. germanica* and *I. florentina*), in which they accumulate during storage. They have been identified as

homologues of the ionones, cyclogeranyl acetonides easily accessible from abundant natural citral (33). Irone, as isolated, is a mixture of α-, β- and γ-irones, with each having the same molecular formula. Petro et al. (34) have prepared porous carbonaceous adsorbent by carbonization of saccharose in silica gel pores, followed by leeching out of the silica matrix. The product of pyrolysis was then deactivated by hydrogenation. A capillary HPLC using this preparation in isocratic mode and UV detection (254 nm) enabled the separation of cis and trans isomers of *α-irone* [CASRN: 79-69-6] (4-(2,5,6,6-tetramethyl-2-cyclohexen-1-yl)-3-butene-2-one).

Neoxanthin

The allenic carotenoid (3S, 5R, 6R, 3′S, 5′R, 6′S)-*neoxanthin* is abundant in higher plants and several algal classes. It was early associated with photosynthetic activity in euglenophytes and green algae, and has been isolated from light harvesting complexes of various algae. In higher plants, the presence of neoxanthin has been reported in the thylakoid membranes of spinach, in photosystem I in tobacco, maize and cotton leaves and in photosystem II in *Arabidopsis thaliana*. Strand et al. (35) have reisolated 9′*Z*-(3S, 5R, 6R, 3′S, 5′R, 6′S)-neoxanthin from spinach, and then submitted this compound to photoinduced stereomutation in the presence of iodine or diphenyl diselenide at conditions that did not involve isomerization of the allenic bond. The six individual geometrical isomers, all-*E*, 9*Z*, 9′*Z*, 13*Z*, 13′*Z*, 15*Z* and three minor di-*Z*-isomers present in the mixture were then characterized using three different HPLC systems, Vis spectral data (λ_{max} shifts, cis-peak intensities), ^{1}H NMR and reversibility tests.

Other Natural Compounds of Interest

Sokoliess et al. (23) showed how silica phases modified with various types of calixarenes and resorcinarenes (within a HPLC methodology) were able to separate geometric cis-trans isomers of isoeugenol [CASRN: 97-54-1] (2-methoxy-4-(1-propenyl)-phenol) and citral [CASRN: 5392-40-5] (3,7-dimethyl-2,6-octadienal). Citral from natural sources is a 2:1 mixture of geranial ((E)-citral) and neral ((Z)-citral). The results gained from this approach were confronted with those collected using a conventional reversed-phase column.

FATTY ACIDS AND VITAMINS

Fatty Acids

Conjugated linoleic acids (CLA) are a group of positional and geometric isomers of octadecadienoic acid (Fig. 1). Various animal models have

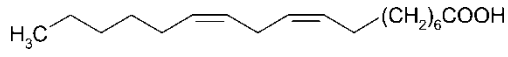


Figure 1. Structure of linoleic acid. CASRN: 60-33-3. (Z,Z)-9,12-octadecadienoic acid.

demonstrated that CLA are anticarcinogenic, hypolipidemic and antiatherosclerotic. CLA have also been shown to enhance immune functions and reduce fat accumulation while they increase muscle and bone mass. These isomers in commercial samples have been separated using gas chromatography (36, 37) performed a derivatization reaction with 4-methyl-1,2,4-triazoline-3,5-dione, thus obtaining derivatives suitable for a subsequent GC-MS/^{13}C-NMR analysis.

Jakubowski et al. (38) investigated fatty acid composition and physical properties of soybean oil during the 3-5 h process of industrial oil hydrogenation. The cis and trans composition was determined through GC and HPLC techniques. Kitayama et al. (39) performed a catalytic hydrogenation of linoleic acid and analyzed the products of this reaction using capillary gas-liquid chromatography, the capillary column being coated with isocyanopropyl trisilphenylene siloxane (TC-70). Various different species were found, including trans-dienoic and conjugated dienoic isomers. Yang et al. (40) have studied the incorporation pattern of conjugated linoleic acids isomers into the egg yolk of hens in relation to that in the diet; the thus resulting fatty acid analysis was achieved by using gas-liquid chromatography/FID subsequent to acid-catalyzed methylation, thin-layer chromatography and silver ion HPLC (Ag-HPLC), that enabled the separation of individual conjugated linoleic acid methyl esters.

Retinoic Acid, Tocopherol and Carotene Derivatives

Retinoic acid and other retinoids are an important class of compounds which have proven to be involved in the regulation and control of several physiological processes, including for example epithelial cell growth and differentiation and vision. Retinoids also have many therapeutic indications. It has to be mentioned that the biological activity of the all-trans and the various cis isomers varies, therefore highlighting the importance of a convenient separation and identification of these isomers.

Retinoids are unstable compounds, they are sensitive to heat, oxygen and light and can undergo isomerization. Bempong et al. (41) used a normal phase LC-MS methodology to determine the degradation products of both all-trans and 13-cis retinoic acids after exposure to air and light. He found 2 different types of degradation reaction: cis-trans isomerization and autooxidation reaction. Strohschein et al. (42) have investigated the use of a C_{30} *vs.* a C_{18} column (LC) for the separation of cis/trans isomers of tretinoin, the

detection being enabled by NMR coupling. Even given the fact that C_{30} columns have a better ability to distinguish between cis/trans isomers of carotenoids than C_{18} columns, a coelution of two isomers on the former could not be avoided. Another approach, employing a reversed-phase HPLC-DAD (Diode Array Detector) methodology for the analysis of retinol/tocopherol/carotene derivatives in human plasma, has also been described (43). All-trans-α-carotene and all-trans-β-carotene were identified by retention time comparisons with standards, whereas the geometrical isomers of β-carotene were identified through HPLC generated UV-Vis absorption spectra. The authors finally suggested that a better specificity of detection could be yielded by using a mass spectrometer connected in-line. To finish, other HPLC methods were performed (44, 45) with calcium hydroxide as a polar stationary phase to resolve β-carotene-isomers in the normal phase mode.

DISCUSSION

In this paragraph, we will try to provide some explanations regarding mechanistic approaches for some of the techniques described in the first three paragraphs. The information mentioned below must not be considered as an absolute one, since some of the mechanisms involved are yet poorly understood.

HPLC Techniques

Conventional HPLC Approaches

Normal-phase HPLC has been used, for example, for the separation of doxepin (7), flupentixol (25) and retinoids isomers (41), and it remains a widespread method in the European Pharmacopoeia (46) (Table 3).

Reversed-phase and reversed-phase ion-pair HPLC methodologies are common techniques, and they have been applied to a broad range of compounds (clomiphene, dosulepin, tamoxifen, clopenthixol, retinoids, neoxanthin, etc.). However, it has to be mentioned in both cases that mechanism of isomer separation lacks detailed study. According to the traditional theory of partition chromatography, differences during the solvatation of analytes (solvation layer) in the mobile phase may explain the isomer's behaviors.

Alkyl C_{30} Stationary Phases (Reversed-Phase HPLC)

Amongst reversed-phase HPLC approaches, alkyl C_{30} phases are the longest chain of monomeric stationary phases available on the market; this kind of

Table 3. Monographs dealing with Z-E isomerism characterization in the European Pharmaceopoeia, 5th edition (46)

DCI	Method used for the determination of (Z)-(E) isomers	Limit (%)
Chlorprothixene hydrochloride	Ion-pair RP-HPLC (R)	E: NMT 2.0
Cinnarizine	RP-HPLC (R)	Z: NMT 0.25
Clomiphene citrate	Normal-phase HPLC (I)	Z: 30.0-50.0
Dinoprost trometamol	RP-HPLC (R)	E: NMT 2.0
Dinoprostone	RP-HPLC (R)	E: NMT 1.5
Dosulepin hydrochloride	RP-phase HPLC (R)	Z: NMT 5.0
Doxepin hydrochloride	RP-HPLC (I)	Z: 13.0-18.5
Flupentixol dihydrochloride	Normal-phase HPLC (I)	Z: 42.0-52.0
Isotretinoin	RP-HPLC (R)	2E,4E,6E,8E: NMT 2.0 sum of others: NMT 0.5
Maleic acid	Normal-phase TLC (I)	E: NMT1.5
Morantel hydrogen tartrate for veterinary use	RP-HPLC (R)	Z: NMT 0.5
Pyrantel embonate	Normal-phase HPLC (R)	Z: NMT 0.5
Sulindac	Normal-phase HPLC (R)	E: NMT 0.5
Tamoxifen citrate	RP-HPLC (R)	E: NMT 0.3
Tretinoin	RP-HPLC (R)	2Z,4E,6E,8E: NMT 2.0 sum of others: NMT 0.5
Ubidecarenone	Normal-phase HPLC (I)	Z: NMT 0.5
Zuclopenthixol decanoate	RP-HPLC (I)	E: NMT 1.25

The compounds have been sorted by alphabetical order. The "R" letter indicates that the Z- (respectively E-) isomer is determined within an assay among other related substances; the "I" letter in turn indicates that the Z- (respectively E-) isomer is determined in an independent assay. NMT = not more than.

column has been successfully used for the separation of cis-trans carotenoid isomers. This unique separation behavior can be related to the exceptional shape selectivity of the C_{30}-phase. Solid-state NMR investigations have indicated that the selectivity results from highly ordered alkyl chains enabling molecular shape recognition for carotenoids and tocopherols (47).

Porous Graphitic Columns and Porous Pyrolytic Carbon Columns

The use of porous graphitic columns (PGC) and porous pyrolytic carbon columns (PPCC) has only been described for a few compounds, including isomers of dosulepin (5) and those of the experimental Eli Lilly & Co.

product LY 170680 (29). Both high specific surface area ($120\ m^2 \cdot g^{-1}$ for PGC) and a special morphology of the surface (sponge-like or flat crystalline, energetically homogeneous surface, respectively) make pyrolytic or graphitic carbon adsorbents sensitive not only to the chemical nature of analytes, but also to their size, shape and position of chemical moieties (34). These unique separation properties could be explained by a combination of various kinds of selective interactions between solutes, mobile phases and stationary phases (electron donor-acceptor interactions, hydrogen bonding, etc.). However, the largest contribution to adsorption on the carboneous surface seems to be related to the dispersion forces of the sorbate molecules (34).

PGC is a form of graphite whose surface consists of delocalized electrons which are capable of interacting with the electrons from injected solutes (π-π interaction). Kiselev and co-workers pioneered the use of PGC as an adsorbent in liquid chromatography (48). Knox and Gilbert (49) described a novel form of carbon, called "porous glassy carbon" and improved its preparation and chromatographic performance in 1986. Structural studies indicated that the carbon produced was graphitic, and not glassy, hence its name PGC (50, 51). PGC is an extremely strong adsorbent (51), because of its flat crystalline surface (52). PGC materials, with minimal active sites on the edge of graphite sheets, have an energetically homogeneous surface (34, 53). This kind of phase is often compared to C_{18} silicas and described as a stronger hydrophobic sorbent (50). However, the retention mechanism appears to be different compared to the RP-bonded silicas (54), and PGC also shows a specific behavior face to solvent strength, which has been demonstrated to be solute-dependent (55, 56). Colin and Guiochon (57) showed that comparisons between the eluotropic strength of solvents used with classical RP-18 stationary phase and carbon adsorbent could not be made because of the specific nature of this chromatographic support; nevertheless, big, bulky and highly polarizable molecules such as carbon tetrachloride or tetrahydrofuran, whatever the solute used on a PGC material, are to be considered as strong solvents on carbon adsorbent (57). When considering for example the isomers of LY 170680 (Eli Lilly & Co.), the different spatial arrangement of the π electrons in each of the isomers would cause the interaction between these and the delocalized surface electrons to be slightly modified for each isomer and hence effect a separation (29). Specific features of PGC columns include, besides an excellent selectivity, an improved stability and they can be used throughout the complete pH range (0–14) without deterioration (55).

Concerning pyrolitic PPC columns, it is still known that their sponge-like surface exhibits a mixed behavior owing to the presence of both polar and non-polar sites (presence of aromatic and oxygen-containing chemical moieties on the surface). PPC's behavior therefore can be sometimes referred to either normal-phase or reversed-phase HPLC. The separation mechanism of irone isomers (34) has been briefly explained using differences in the chemical structure of the molecules (planarity/size of the contact surface with the stationary phase).

Chiral Stationary Phases

Liquid chromatography with a *chiral* (i.e., optically active) stationary phase rapidly separates enantiomers, which are, as previously mentioned, nonsuper-imposable mirror images of the same compound.

Even if this kind of columns was not primarily intended to be used in this context, there is still a great deal of interest regarding its use in cis-trans isomers separation. For example, Pan et al. (58) described the separation and subsequent identification of both cis and trans isomers of butene-1,4-diol and lafutidine (a second generation histamine-H_2-receptor antagonist) on 2 types of chiral columns ((S,S)-Whelk-O 1/ChiraSpher), whereas Schmid et al. (59) discussed the use of an optically active poly(tritylmethacrylate) chiral stationary phase supported on Nucleosil to separate some stereoisomers of (Z)- and (E)-vitamin K1.

Amongst these phases, we will further distinguish between 2 classes: calixarene-/resorcinarene-bonded and cyclodextrin-bonded materials.

Calixarene- and Resorcinarene-Bonded Stationary Phases

In the field of calixarene- or resorcinarene-bonded silica stationary phases, Sokoliess et al. (8) discussed potential mechanisms of separation based on the size of both the cavities (width, depth) and analytes, the (Z)- and (E)-forms having a different spatial organization; the flexibility of the cavities has also been evoked (Fig. 2a, b, c).

Among the columns tested, the amount of organic modifier (methanol, acetonitrile) was supposed to be dependent from the substitution of the calixarenes, thus possibly leading to strong differences in selectivities of the stationary phases.

The resorcinarene-bonded phase proved to be very selective for the separation of the isomers of the thioxanthene containing a hydroxyethyl substituted piperazinyl group, such as flupentixol and clopenthixol; this could be due to an interaction between the hydroxyl group of the analyte and phenol groups of the resorcinarene. On both calixarene-bonded and RP-C_{18} stationary phases, where such interactions are not given, smaller separation factors were obtained.

For the separation of chlorprothixene, similar selectivities on calixarene as well as on RP-C_{18} stationary phases were yielded, but the resolution on the RP-phase was found to be higher. A kinetic effect has been discussed, pointing out the fact that the inclusion of parts of the analytes into the cavities of calixarene-bonded phases needs a longer time than the interaction of analytes with the hydrocarbon chains of a RP-C_{18} phase.

To finish, doxepin isomers were best separated on stationary phases containing small calixarenes without para-tert-butyl groups; this was supposed to be due to a better interaction and a good fitting of aromatic parts of the analytes and the aromatic units of these calixarenes.

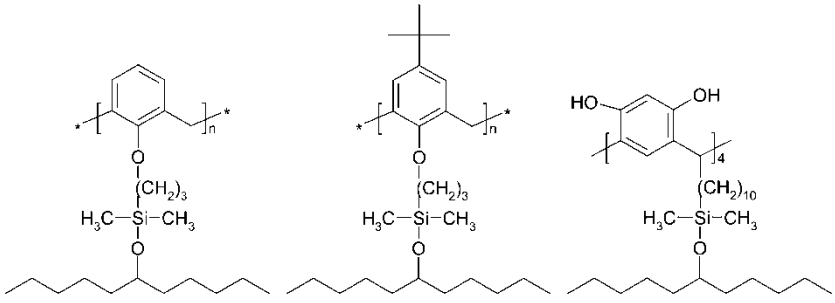


Figure 2. (a) Structure of a silica-bonded calix[n]arene phase (Caltrex AI, II, III, Synaptec, Germany; n = 4, 6, 8 respectively). (b) Structure of a silica-bonded para-tert-butylcalix[n]arene phase (Caltrex BI, II, III, Synaptec, Germany; n = 4, 6, 8 respectively). (c) Structure of a silica-bonded resorcinarene phase (RES, Synaptec, Germany).

Cyclodextrin-Derivatives Columns

Since the isolation of β-cyclodextrin by Villiers (earlier designated as "cellulosines"; (60)) there has been a tremendously growing number of publications covering this subject. Cyclodextrins are a family of torus-shaped, naturally occurring cyclic oligosaccharides composed of six (α-), seven (β-, the most widely used one), eight (γ-) or nine (δ-cyclodextrin) α-1,4 linked D-glucopyranose units per molecule (61, 62) (see Figure 3).

While the exterior of the molecule is hydrophilic, its relatively non-polar central cavity (63) may selectively include molecules of various species. Cyclodextrins can either be bonded to the silica support to make an original stationary phase or be added to the mobile phase as chiral selectors.

Cyclodextrin-derivative columns are a versatile class of chiral stationary phases. The hydroxyls can be capped with chemical moieties such as pentyl ($-C_5H_{11}$) or trifluoroacetyl ($-COCF_3$) to decrease the polarity of the faces (64).

Each enantiomer of a chiral analyte has a different affinity for the cavity inside cyclodextrins. This type of methodology could therefore also be applied to the separation of geometric isomers (separation of cis-trans isomers of α-tocotrienol by HPLC using a permethylated β-cyclodextrin phase (65); separation of (E)- and (Z)-doxepin with β-cyclodextrin-bonded stationary phase (66); separation of tamoxifen geometric isomers and metabolites by bonded-phase β-cyclodextrin chromatography (67).

Other Types of Stationary Phases Used in HPLC

Other kinds of stationary phases employed for (Z)-(E) isomer separation also include monolithic columns (Chromolith columns, determination of cis- and

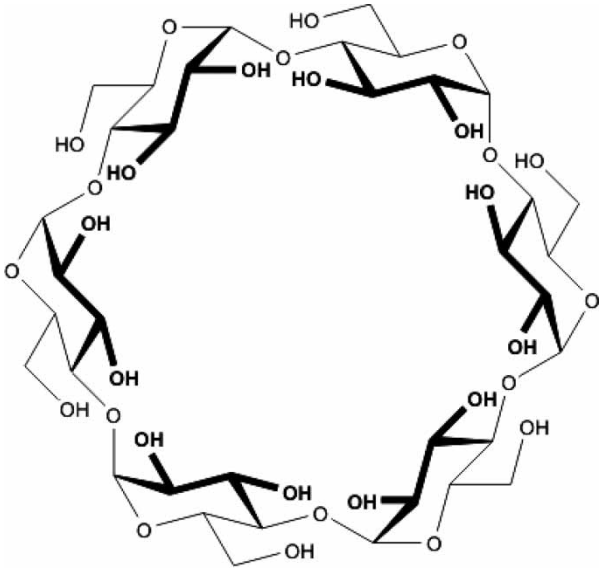


Figure 3. Molecular structure of α-cyclodextrin.

trans-resveratrol in wine, gradient mode without additives in the mobile phase (68)). Monolithic silica columns are packed with a single piece of silica gel into a straight rod of highly porous silica with a bimodal pore structure (69, 70). Due to their rigid and porous structure, they enable higher solvent flow rates, shorter analysis duration and a fast equilibration. Due to the low back pressure, it is possible to connect several columns (71).

Calcium hydroxide columns HPLC (44, 45) and silver-ion HPLC (7) were also performed. The influence of sulfur-aromatic interactions on the chromatographic separation behavior of hybrid RP-phases containing thiol-groups and/or embedded sulfide-groups has been investigated using various chromatographic column tests (72). Several types of sulfide groups-RP (S-RP) were prepared by Horak and Lindner (72) (C_3-S-C_{18}/SH, C_3-S-C_{14}/SH, C_3-S-C_8/SH, C_3-SH) and utilized for the separation of cis-trans isomers of stilbene, stilbene oxide and resveratrol. The different retention times and shape selectivities observed within this study were discussed considering the polarity (hydrophobic areas and interactions) and the availability of additional hydrogen bonding sites of the analytes. In general, the separation properties of these S-RP phases may be placed between that of conventional brush-type RP phases and polymeric shape-selectivity-PAH (Poly Aromatic Hydrocarbon)-phases according to the authors (72).

Fluorinated columns (73) could also provide better recognition of geometrical isomers and epimers due to a less solvophobic interaction than on classic C_{18}-columns and the rigid molecular structure of the fluorocarbon chain.

Subcritical and Supercritical Fluid Chromatography (SFC)

In supercritical fluid chromatography (SFC), it has been shown that the three-dimensional structure of the stationary phase depends on the mobile phase adsorption into the bonded one. This is why varying the percentage of modifier may have an influence on the selectivity between compounds having either a linear or a bent molecular structure (in case of cis-trans isomerism of β-carotene; (74)).

Recently, several monolithic stationary phases (including YMC and Chromolith columns) were used to achieve the separation of β-carotene isomers in subcritical chromatography (74). Other SFC attempts, for the most part on β-carotene or vitamin A derivatives, have been made either in the 1990s or the late 1980s in the field of cis-trans isomer separation (75–77).

Capillary Electrophoresis

In capillary electrophoresis analysis, many factors can be varied in order to influence separation. These factors include buffer composition, ionic strength, pH, applied voltage and capillary dimensions. In addition, additives such as organic solvents, cyclodextrins and surfactants can be used to affect separation.

Aqueous and Non-Aqueous Capillary Electrophoretic Separations Using Cyclodextrins or Other Compounds as Chiral Selectors

Capillary electrophoretic separation employing various cyclodextrin derivatives has already been described for the isomers of doxepin (9) as well as those of clomiphene (15, 16). In several cases, various cyclodextrins have already been used for the separation of positional and optical isomers in capillary electrophoresis and micellar electrokinetic chromatography (MEKC) (78, 79). The literature provides examples in which the resolution value shows a maximum in the function of cyclodextrin, in term of size, concentration and/or charge, and this maximum does not require more than one type of interaction (80). The derivatized cyclodextrin with ionic substituents can separate even neutral compounds (81). Mixtures of various cyclodextrin derivatives were also used in order to yield better separation characteristics. One cyclodextrin acts as a chiral selector, whereas the other only acts as a solubilizing agent or a migration time-reducing additive in some instances (82, 83).

Juvancz et al. (15) developed a capillary electrophoretic separation of clomiphene isomers using various derivatives of cyclodextrins as additives and investigated the effect of several parameters (background electrolytes, pH and type and concentration of cyclodextrins). Other attempts, using SDS above its micelle concentration, yielded no promising results. Bempong and

Honigberg (16) also investigated the factors affecting the resolution between the two geometric isomers of clomiphene. The electropherogram showed no resolution when no cyclodextrin derivative was added into the medium.

Sokoliess et al. (11) used 6 various soluble calixarenes and resorcinarenes as additives in NACE, and discussed the influence of several parameters on the selectivity. Within this framework, the concentration of the calixarene, the apparent pH value as well as the organic modifier were the most important factors. Sokoliess et al. (11) confirmed the principal results of Hansen et al. (17) and highlighted the fact that the potential of calixarenes for thioxanthene derivatives analysis had to be seen at higher apparent pH values. Independently on the nature of calixarene, all (Z)-isomers had a faster migration rate than the (E)-isomers. Hansen et al. (17) already provided some explanation by saying that this is due to a tighter "packing" of the molecule as a result of molecular interactions between groups in the side chain and the functional groups on the ring system and solvation effects. Electrostatic interactions, as well as hydrophobic effects and flexibility of the cavity's upper rim, have also been mentioned within this context.

Capillary Electrophoretic Separations Without Chiral Selectors

NACE using acidic media (17) has been shown to provide fast and efficient separations of cationic cis-trans isomers and diastereoisomers, especially for less water soluble substances, without using additives like surfactants, cyclodextrins or other complexing agents to the electrophoresis medium. In this case, the separation is highly dependent on the solvent, as well as on the type and concentration of the electrolytes used. The team discussed the dependence of changes in pH* (apparent pH value) and in the nature and concentration of the electrolyte on the separation selectivity, investigating a great amount of electrophoresis media. This article provides evidence for a non-dependence of separation on differences in the pK_a value of the substances, but rather for a dependence on the apparent volume of the conformers, which is a result of intramolecular interactions and solvatation.

CONCLUSIONS

(Z)-(E) isomerism remains an important topic especially when considering pharmaceutical compounds, since these isomers can have different biological, pharmacological or toxicological properties. The separation and subsequent detection of (Z)/(E)-isomers of both synthetic and natural compounds is a challenge for the modern analyst, and encompasses a large variety of methods. HPLC-based methods are the most numerous ones, even if some electrophoretic capillary studies and SFC methodologies have been developed.

Among the HPLC methods, it has to be mentioned that porous graphitic carbon (PGC) columns, even if their use is yet not widespread, are exhibiting interesting characteristics (wide pH range stability, excellent selectivity and improved thermal stability) along with fluorinated columns. Alkyl C_{30} stationary phases (RP-HPLC) can be successfully used for the separation of carotenoids derivatives, whereas monolithic columns, due to their rigid and porous structure, enable higher solvent flow rates, shorter analysis duration, fast equilibration and offer the possibility to connect several columns. The use of calcium hydroxide- as well as silver-ion HPLC remains confidential.

In HPLC as well as in CE techniques, the use of chiral selectors (cyclodextrin, calixarene and resorcinarene derivatives), which can either be bonded to the solid support (HPLC) or be added to the mobile phase/separation medium (HPLC/CE) represents another valuable alternative to achieve (Z)-(E) isomer separation.

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REFERENCES

1. IUPAC (1997) *Compendium of Chemical Terminology*, 2nd Ed; Blackwell Science: Boston.
2. Cahn, R.S., Ingold, C.K., and Prelog, V. (1966) Specification of molecular chirality. *Angew. Chem.*, 78: 413–447.
3. FDA's policy statement for the development of new stereoisomeric drugs. U.S. Food and Drug Administration/CDER, published May 5, 1992, updated July 6, 2005.
4. Coudore, F., Ardid, D., Eschalier, A., Lavarenne, J., and Fialip, J. (1992) High-performance liquid chromatographic determination of amitryptiline and its main metabolites using a silica column with reversed-phase eluent. Application in mice. *J. Chromatogr. B*, 584: 249–255.
5. Pawlak, Z., Kay, D., and Clark, B.J. (1990) Assay of dothiepin hydrochloride and its isomers by liquid chromatography. *Anal. Proc.*, 27: 16–18.
6. Hrdina, P.D., Bakish, D., Swenson, S., and Lapierre, Y.D. (1990) Cis- and trans-isomers of doxepin and desmethyldoxepin in the plasma of depressed patients treated with doxepin. *Ther. Drug Monit.*, 12: 129–133.
7. Yan, J.H., Hubbard, J.W., McKay, G., and Midha, K.K. (1997) Stereoselective and simultaneous measurement of cis- and trans-isomers of doxepin and N-desmethyldoxepin in plasma or urine by high-performance liquid chromatography. *J. Chromatogr. B*, 691: 131–138.
8. Sokoliess, T., Menyes, U., Roth, U., and Jira, T. (2002) Separation of cis- and trans-isomers of thioxanthene and dibenz[b,e]oxepin derivatives on calixarene- and

resorcinarene-bonded high-performance liquid chromatography stationary phases. *J. Chromatogr. A*, 948: 309–319.
9. Hu, Q., Tian, S., Che, B., and Zhang, L. (2001) Determination of doxepine hydrochloride by capillary electrophoresis. *Zhongguo Yaoxue Zazhi*, 36: 842–844.
10. Hu, Q., Tian, S., and Xu, M. (2002) Determination of doxepine hydrochloride by nonaqueous capillary electrophoresis. *Zhongguo Yaoxue Zazhi*, 37: 620–622.
11. Sokoliess, T., Gronau, M., Menyes, U., Roth, U., and Jira, T. (2003) Separation of (Z)- and (E)-isomers of thioxanthene and dibenz[b,e]oxepin derivatives with calixarenes and resorcinarenes as additives in nonaqueous capillary electrophoresis. *Electrophoresis*, 24: 1648–1657.
12. Baustian, C.L. and Mikkelson, T.J. (1986) Analysis of clomiphene isomers in human plasma and detection of metabolites using reversed-phase chromatography and fluorescence detection. *J. Pharm. Biomed. Anal.*, 4: 237–246.
13. Mikkelson, T.J., Kroboth, P.D., Cameron, W.J., Dittert, L.W., Chungi, V., and Manberg, P.J. (1986) Single-dose pharmacokinetics of clomiphene citrate in normal volunteers. *Fertil. Steril.*, 46: 392–396.
14. Urmos, I., Benko, S.M., and Klebovich, I. (1993) Simple and rapid determination of clomiphene cis and trans isomers in human plasma by high-performance liquid chromatography using on-line post-column photochemical derivatization and fluorescence detection. *J. Chromatogr. B*, 617: 168–172.
15. Juvancz, Z., Urmos, I., and Klebovich, I. (1996) Capillary electrophoretic separation of clomiphene isomers using various cyclodextrins as additives. *J. Capillary Electroph.*, 3: 181–189.
16. Bempong, D.K. and Honigberg, I.L. (1996) Multivariate analysis of capillary electrophoresis separation conditions for Z-E isomers of clomiphene. *J. Pharm. Biomed. Anal.*, 15: 233–239.
17. Hansen, S.H., Bjornsdottir, I., and Tjornelund, J. (1997) Separation of cationic cis-trans (Z-E) isomers and diastereoisomers using non-aqueous capillary electrophoresis. *J. Chromatogr. A*, 792: 49–55.
18. Chen, Y., Wang, Y., and Wang, Z. (1987) Reversed ion-pair chromatography for tamoxifen citrate. *Sepu*, 5: 388–393.
19. Husain, S., Alvi, S.N., and Rao, R.N. (1994) Separation and determination of *E* and Z-isomers of tamoxifen by ion-pair high-performance liquid chromatography. *Anal. Lett.*, 27: 2485–2497.
20. Manns, J.E., Hanks, S., and Brown, J.E. (1998) Optimized separation of E- and Z-isomers of tamoxifen, and its principal metabolites using reversed-phase high-performance liquid chromatography. *J. Pharm. Biomed. Anal.*, 16: 847–852.
21. Snyder, L.R. (1978) Classification of the solvent properties of common liquids. *J. Chrom. Sci.*, 16: 223–224.
22. Zhu, S., Niu, C., and Liu, Q. (1998) Separation and determination of Z, E isomers of toremifen by high performance capillary electrophoresis. *Yaowu Fenxi Zazhi*, 18: 250–252.
23. Sokoliess, T., Menyes, U., Roth, U., and Jira, T. (2001) Cup-type structures separate cis/trans isomers. *Laborpraxis*, 25: 28–30.
24. Hansen, B.B. and Hansen, S.H. (1994) Determination of zuclopenthixol and its main N-dealkylated metabolite in biological fluids using high-performance liquid chromatography with post-column photochemical derivatization and fluorescence detection. *J. Chromatogr. B*, 658: 319–325.
25. Walter, S., Bauer, S., Roots, I., and Brockmoller, J. (1998) Quantitation of the antipsychotics flupentixol and haloperidol in human serum by high-performance liquid chromatography with ultraviolet detection. *J. Chromatogr. B*, 720: 231–237.

26. Severin, G. (1987) Comprehensive high-performance liquid-chromatography methodology for the determination of thiothixene in bulk drug, finished product, and dissolution testing samples. *J. Pharm. Sci.*, 76: 231–234.
27. Dilger, C., Salama, Z., and Jaeger, H. (1988) Improved high-performance liquid-chromatographic method for the determination of thiothixene in human serum. *Arzneimittelforsch*, 38: 1522–1525.
28. Karpas, Z., Stimac, R.M., and Rappoport, Z. (1988) Differentiating between large isomers and derivation of structural information by ion mobility spectrometry/mass spectrometry techniques. *Int J. Mass Spectrom*, 83: 163–175.
29. Fish, B.J. (1993) Use of a porous graphite carbon column to separate cis and trans isomers in a novel antiasthma compound. *J. Pharm. Biomed. Anal.*, 11: 517–521.
30. Adam, W., Halasz, J., Jambor, Z., Levai, A., Nemes, C., Patonay, T., and Toth, G. (1996) Synthesis and stereochemistry of the epoxides of 2-arylmethylidene-1-tetralones. *J. Chem. Soc.—Perkin Trans.*, 1 (4): 395–400.
31. Zawisza, I., Bilewicz, R., Luboch, E., and Biernat, J.F. (1998) Voltammetric recognition of cis (Z) and trans (E) isomers of azobenzene and azo-crown ethers. *Supramol. Chem.*, 9: 277–287.
32. Lange, F.J., Leuze, M., and Hanack, M. (2001) E isomerization of poly-para-phenylenevinylene model compounds. *J. Phys. Org. Chem.*, 14: 474–480.
33. Jaenicke, L. and Marner, F.J. (1990) The irones and their origin. *Pure & Appl. Chem.*, 62: 1365–1368.
34. Petro, M., Belliardo, F., Novak, I., and Berek, D. (1998) Use of porous pyrolytic carbon for analytical and microscale high-performance liquid chromatographic bioseparations. *J. Chromatogr. B*, 718: 187–192.
35. Strand, A., Kvernberg, K., Karlsen, A.M., and Liaaen-Jensen, S. (2000) Carotenoids in higher plants. 15. Geometrical *E*/Z isomers of (6R)- and (6S)-neoxanthin and biological implications. *Biochem. Syst. Ecol.*, 28: 443–455.
36. Christie, W.W., Dobson, G., and Gunstone, F.D. (1997) Isomers in commercial samples of conjugated linoleic acid. *Lipids*, 32: 1231.
37. Precht, D. and Molkentin, J. (1997) Trans-geometrical and positional isomers of linoleic acid including conjugated linoleic acid (CLA) in German milk and vegetable fats. *Fett/Lipid*, 99: 319–326.
38. Jakubowski, A., Pilat, K., Grzeskiewicz, S., and Braczko, M. (1997) Geometric and positional isomerization during hydrogenation of soybean oil in Poland. *Tluszcze Jadalne.*, 32: 25–44.
39. Kitayama, Y., Takahashi, M., Sugiyama, H., Kodama, T., and Okamura, M. (1998) Gas-liquid chromatographic analysis of geometrical and positional octadecenoic and octadecadienoic acid isomers produced by catalytic hydrogenation of linoleic acid on Ir/Al_2O_3. *J. Am. Oil Chem. Soc.*, 75: 27–32.
40. Yang, L., Huang, Y., James, A.E., Lam, L.W., and Chen, Z.Y. (2002) Differential incorporation of conjugated linoleic acid isomers into egg yolk lipids. *J. Agric. Food Chem.*, 50: 4941–4946.
41. Bempong, D.K., Honigberg, I.L., and Meltzer, N.M. (1995) Normal phase LC-MS determination of retinoic acid degradation products. *J. Pharm. Biomed. Anal.*, 13: 285–291.
42. Strohschein, S., Schlotterbeck, G., Richter, J., Pursh, M., Tseng, L.H., Händel, H., and Albert, K. (1997) Comparison of the separation of cis/trans isomers of tretinoin with different stationary phases by liquid chromatography-nuclear magnetic resonance coupling. *J. Chromatogr. A*, 765: 207–214.
43. Lane, J.R., Webb, L.W., and Acuff, R.V. (1997) Concurrent liquid chromatographic separation and photodiode array detection of retinal, tocopherols,

all-trans-α-carotene, all-trans-β-carotene and the mono-cis isomers of β-carotene in extracts of human plasma. *J. Chromatogr. A*, 787: 111–118.
44. Schmitz, H.H., Schwartz, S.J., and Catignani, G.L. (1994) Resolution and quantitation of the predominant geometric β-carotene isomers present in human serum using normal-phase HPLC. *J. Agric. Food Chem.*, 45: 2746–2750.
45. Schmitz, H.H., Emenhiser, C., and Schwartz, S.J. (1995) HPLC separation of geometric carotene isomers using a calcium hydroxide stationary phase. *J. Agric. Food Chem.*, 43: 1212–1218.
46. European Pharmacopoeia, 5th ed. (2005) EDQM, Strasbourg, France.
47. Pursch, M., Strohschein, S., Handel, H., and Albert, K (1996) Temperature-dependent behavior of C30 interphases. A solid-state NMR and LC-NMR study. *Anal. Chem.*, 68: 386–393.
48. Kiselev, A.V., Nikitin, Y.S., Frolov, I.I., and Yashin, Y.I. (1974) Problems of selectivity and efficiency in liquid-solid chromatography. *J. Chromatogr.*, 91: 187–200.
49. Knox, J.H. and Gilbert, M.T. Preparation of porous carbon. UK Patent 7939449 (1980); US patent 4,263,268 (1981); FRG patent P294 6688-4 (1980).
50. Knox, J.H., Kaur, B., and Millard, G.R. (1986) Structure and performance of porous graphitic carbon in liquid chromatography. *J. Chromatogr.*, 352: 3–25.
51. Kaur, B. (1986) Porous graphitic carbon: a new material for high performance liquid chromatography. PhD Thesis, University of Edinburgh: UK.
52. Unger, K.K. (1983) Porous carbon packings for liquid chromatography. *Anal. Chem.*, 55: 361–375A.
53. Wan, Q.H., Shaw, P.N., Davies, M.C., and Barett, D.A. (1995) Chromatographic behavior of positional isomers on porous graphitic carbon. *J. Chromatogr. A*, 697: 219–227.
54. Hennion, M.C., Coquart, V., Guenu, S., and Sella, C. (1995) Retention behaviour of polar compounds using porous graphitic carbon with water-rich mobile phases. *J. Chromatogr. A*, 712: 287–301.
55. Kaur, B. (1989) The use of porous graphitic carbon in high performance liquid chromatography. *LC-GC Int.*, 3: 41–48.
56. Applications notes, Hypersil, S.A., Chadwick Road, Astmoor, Runcorn, Cheshire WA7 1PR, England.
57. Colin, H. and Guiochon, G. (1982) The solvent eluotropic strength on carbon adsorbents. *Chromatographia*, 15: 133–139.
58. Pan, C.X., Xu, X.Z., He, H.M., Cai, X.J., and Zhang, X.J. (2005) Separation and identification of cis and trans isomers of 2-butene-1,4-diol and lafutidine by HPLC and LC-MS. *J. Zhejiang. Univ. SCI.*, 6B: 74–78.
59. Schmid, R., Antoulas, S., Ruettimann, A., Schmid, M., Vecchi, M., and Weiser, H. (1990) Synthesis of all four stereoisomers of (E)-vitamin K1 (phylloquinone), analysis of their diastereoisomeric and enantiomeric purities and determination of their biopotencies. *Helv. Chim. Acta.*, 73: 1276–1299.
60. Villiers, A. (1891) Sur la fermentation de la fécule par l'action du ferment butyriqué. *Compt. Rend.*, 112: 536–538.
61. Szejtli, J. (1982) *Cyclodextrins and Their Inclusion Complexes*; Akademiai Kiado: Budapest.
62. Schardinger, F. (1911) The formation of crystallin polysaccharides (dextrins) from starch paste by microorganisms. *Zentralbl. Bakteriol. Parasintenk. Abt. 2*, 29: 188.
63. Street, K.W., Jr. (1987) Cyclodextrin cavity polarity and chromatographic implications. *J. Liq. Chromatogr.*, 10: 655–662.

64. Berthod, A., Li, W., and Armstrong, D.W. (1992) Multiple enantioselective retention mechanisms on derivatized cyclodextrin gas chromatographic chiral stationary phases. *Anal. Chem.*, 64: 873–879.
65. Drotleff, A.M. and Ternes, W. (1998) Separation and characterization of cis-trans isomers of α-tocotrienol by HPLC using a permethylated β-cyclodextrin phase. *Z. Lebensm. Unters. Forsch.*, 206: 9–13.
66. Piperaki, S., Parissi-Poulou, M., and Koupparis, M. (1993) A separation study of tricyclic antidepressant drugs by HPLC with β-cyclodextrin-bonded stationary phase. *J. Liq. Chromatogr.*, 16: 3487–3508.
67. Armstrong, R.D., Ward, T.J., Pattabiraman, N., Benz, C., and Armstrong, D.W. (1987) Separation of tamoxifen geometric isomers and metabolites by bonded-phase β-cyclodextrin chromatography. *J. Chromatogr.*, 414: 192–196.
68. Vian, M.A., Tomao, V., Gallet, S., Coulomb, P.O., and Lacombe, J.M. (2005) Simple and rapid method for cis- and trans-resveratrol and piceid isomers determination in wine by high-performance liquid chromatography using Chromolith columns. *J. Chromatogr. A*, 1085: 224–229.
69. Nakanishi, K. and Soga, N. (1991) Phase separation in gelling silica-organic polymer solution: systems containing poly(sodium styrenesulfonate). *J. Am. Ceram. Soc.*, 74: 2518–2530.
70. Nakanishi, K. and Soga, N. (1992) Phase separation in silica sol-gel system containing polyacrylic acid. I. Gel formation behavior and effect of solvent composition. *J. Non-Cryst. Solids.*, 139: 1–13.
71. Bamba, T., Fukusaki, E., Nakazawa, Y., and Kobayashi, A. (2004) Rapid and high-resolution analysis of geometric polyprenol homologues by connected octadecylsilylated monolithic silica columns in high-performance liquid chromatography. *J. Sep. Sci.*, 27: 293–296.
72. Horak, J. and Lindner, W. (2004) Investigations on the chromatographic behavior of hybrid reversed-phase materials containing electron donor-acceptor systems. I. Contribution of sulfur-aromatic interactions. *J. Chromatogr. A*, 1043: 177–194.
73. Kamiusuki, T., Monde, T., Yano, K., Yoko, T., and Konakahara, T. (1999) Separation behavior of various organic compounds on branched-polyfluoroalkylsilane coated silica gel columns. *J. Chromatogr. Sci.*, 37: 388–394.
74. Lesellier, E., West, C., and Tchapla, A. (2003) Advantages of the use of monolithic stationary phases for modeling the retention in sub/supercritical chromatography. Application to cis/trans-β-carotene separation. *J. Chromatogr. A*, 1018: 225–232.
75. Schmitz, H.H., Artz, W.E., Poor, C.L., Dietz, J.M., and Erdman, J.W., Jr. (1989) High-performance liquid chromatography and capillary supercritical-fluid chromatography separation of vegetable carotenoids and carotenoid isomers. *J. Chromatogr.*, 479: 261–268.
76. Braumann, U., Handel, H., Strohschein, S., Spraul, M., Krack, G., Ecker, R., and Albert, K. (1997) Separation and identification of vitamin A acetate isomers by supercritical fluid chromatography-^{1}H NMR coupling. *J. Chromatogr. A*, 761: 336–340.
77. Lesellier, E., Gurdale, K., and Tchapla, A. (1999) Separation of cis/trans isomers of β-carotene by supercritical fluid chromatography. *J. Chromatogr. A*, 844: 307–320.
78. Terabe, S., Miyashita, Y., Ishihama, Y., and Shibata, O. (1993) Cyclodextrin-modified micellar electrokinetic chromatography: separation of hydrophobic and enantiomeric compounds. *J. Chromatogr.*, 636: 47–55.
79. Nishi, H. and Terabe, S. (1995) Optical resolution of drugs by capillary electrophoresis techniques. *J. Chromatogr. A*, 694: 245–276.

80. Wren, A.C. and Rowe, R.C. (1992) Theoretical aspects of chiral separation in capillary electrophoresis. I. Initial evaluation model. *J. Chromatogr.*, 603: 235–241.
81. Schmidt, T. and Engelhardt, H. (1993) Charged and uncharged cyclodextrins as chiral selectors in capillary electrophoresis. *Chromatographia*, 37: 475–481.
82. Solar, O.H.J., Brown, R.S., and Loung, T.S.H. (1995) Separation of PAH by capillary electrophoresis with laser-induced fluorescence detection using mixtures of neutral and anionic β-cyclodextrins. *Anal. Chem.*, 67: 3004–3010.
83. Lurie, I.S., Klein, R.F.X., Dal Cason, T.A., Lebelle, M.J., Brenneisen, R., and Weinberger, R.E. (1994) Chiral resolution of cationic drugs of forensic interest by capillary electrophoresis with mixtures of neutral and anionic cyclodextrins. *Anal. Chem.*, 66: 4019–4026.