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Review

Analyzing the Chiral Purity of Pharmaceuticals: The Application of Cyclodextrin-Based Chiral Selectors in Capillary Electrophoresis

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Abstract: The current review provides a focused analysis of the application of capillary electrophoresis (CE) techniques to determine the chiral purity of pharmaceuticals, with a specific emphasis on cyclodextrin- (CD) based chiral selectors (CSs), highlighting advancements, methodologies, and trends in this area as reported in studies published from 2010 to 2024. The review emphasizes CE's evolution as a critical tool in this field, discussing its advantages, such as high efficiency, flexibility, relatively low costs, and minimal environmental impact, which make it well-suited for modern pharmaceutical applications. Additionally, it underscores the importance of CE in meeting stringent regulatory requirements for chiral drug substances. A significant shift in method optimization has occurred in the last ten years, shifting from the traditional One-Factor-at-a-Time (OFAT) strategy to the Design-of-Experiments (DoE) approach; this shift has enabled more systematic and robust method development. Furthermore, a common trend in recent years is the application of Quality-by-Design (QbD) principles in method development and optimization, ensuring higher reliability and efficiency. Additionally, there is an increasing focus on developing CE methods capable of detecting both achiral and chiral impurities simultaneously, which enhances the comprehensiveness of the analysis. This review seeks to guide future research and development in optimizing CE methodologies for pharmaceutical applications.

Keywords: chirality; capillary electrophoresis; enantioseparation; chiral purity; chiral selector; cyclodextrins

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1. Introduction

Chirality is a property of asymmetry, fundamental to our understanding of the natural world, impacting scientific fields as diverse as mathematics, physics, and of course, chemistry. In chemistry, chirality allows us to distinguish between racemic mixtures and pure enantiomers, but this distinction becomes particularly important in the world of pharmaceutical substances, where enantiomers of a chiral drug can exhibit different pharmacological properties. Nature itself is a testament to the importance of chirality in life, with biological molecules being homochiral; for example, all amino acids are levorotatory, and all sugars are dextrorotatory. This intrinsic asymmetry, or chirality, underpins the functionality of these molecules in biological systems [1,2].

It is well known that the desired pharmacological effects of a chiral substance are linked with the activity of a single enantiomer called a eutomer, while the other enantiomer, the distomer, is usually less potent but sometimes can be responsible for the adverse effects of racemic administration [3,4]. One of the most tragic examples of a chiral drug causing problems is thalidomide, which was used in the late 1950s for its hypnotic and anti-nausea properties; thalidomide was administered to pregnant women to combat morning sickness,

but while *R*-thalidomide is an effective sedative that relieves anxiety and induces sleep, *S*-thalidomide is teratogenic and caused severe birth defects in thousands of children born to women who were administered the drug during the first trimester of pregnancy [5].

Although optical isomers possess the same chemical structure and the same physicochemical properties (being distinguished solely by an optical property, specifically the direction in which they rotate the polarized light), they often present differences in terms of pharmacological, pharmacotoxicological, and pharmacokinetic profiles, explained by the fact that the human body is made up of chiral elements, which makes it the most complex and difficult to understand "chiral selector" [6].

The year 1992 marked the pivotal moment for chirality in the pharmaceutical industry when the Food and Drug Administration (FDA) introduced the first specific guidelines for the development and approval of chiral drugs; the European Medicines Agency (EMA) implemented a similar policy in 1994. Both regulatory bodies recommended the development of optically pure enantiomers, although it was not made mandatory. These guidelines enforced strict requirements for the pharmacokinetic, pharmacodynamic, and pharmacotoxicological characterization of both individual enantiomers and the racemic mixture of any chiral substance intended for therapeutic use. This shift significantly impacted on the scientific community, particularly those involved in developing and validating analytical methods for chiral drug analysis [7,8].

Although the FDA and EMA have not mandated the development of optically pure enantiomers, allowing the use of racemic mixtures when appropriate, enantiomerically pure drugs have become the "golden standard" among innovative pharmaceutical companies. Consequently, there has been a significant trend in modern therapy towards the use of optically pure enantiomers, as evidenced by the substantial number of such substances introduced into therapy over the past 20–25 years [9]. In fact, more than 90% of the optically active substances approved by the FDA in the last decade are enantiomerically pure. Additionally, the "chiral switch" phenomenon, where racemic mixtures previously used in therapy are replaced with optically pure enantiomers (specifically the eutomer), was notably prevalent from 1990 to 2010 [10].

When developing a new enantiomerically pure drug, the pharmaceutical industry typically relies on three primary approaches: starting with a pure enantiomer from a natural product (known as the "chiral pool"), employing stereoselective synthesis methods (which may include enzymatic or biological processes), or separating a racemate produced through a non-stereoselective synthesis method ("chiral resolution"). Regardless of the approach, the innovator company is required to provide detailed specifications for the final product that guarantee its stereochemical identity, robustness, quality, and purity [9,11].

It has become clear that developing methods for the enantioselective separation of chiral substances is crucial, both for the pharmaceutical industry and for clinical applications. Understanding the stereoselective biotransformation that chiral substances undergo in the body after administration (stereoselective metabolism) and determining the optical purity of pure enantiomers necessitates the development of modern and efficient analytical methods for enantioseparation. These advancements are essential for ensuring the efficacy and safety of chiral drugs [12,13].

While chromatographic techniques, particularly high-performance liquid chromatography (HPLC), gas chromatography (GC), and supercritical fluid chromatography (SFC), remain the most used techniques for chiral separation, electrokinetic separation methods like capillary electrophoresis (CE) and capillary electrochromatography (CEC) offer several distinct advantages [13]. CE provides high efficiency, fast analysis times, and high resolution for small sample volumes. Furthermore, CE requires minimal amounts of solvents and analytes, significantly reducing both operational costs and environmental impact, in line with the principles of "green" chemistry. Their flexibility in changing chiral selectors (CSs) and rapid method development also contribute to their growing popularity as alternatives to traditional chromatographic methods. CE is also advantageous for its ability to separate compounds based on their charge-to-size ratio, making it highly effective in enantioselec-

tive separations without requiring expensive chiral stationary phases. These advantages underscore the utility of electrokinetic separation techniques in the field of pharmaceutical chiral analysis [14,15].

2. Capillary Electrophoresis in Chiral Separation of Pharmaceuticals

CE has become an enantioseparation method that is gaining ground in the analysis of chiral pharmaceuticals, representing the main alternative to the widely used chromatographic techniques in the pharmaceutical industry and research. CE is a separation technique that relies on the movement of electrically charged particles within a capillary dissolved in an electrolyte solution, driven by the application of an electric field [14].

When we refer to CE, we are not discussing a single analytical method but rather a family of related techniques that operate on similar separation principles; within this family, capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC) are commonly used for the analysis of pharmaceuticals, including those of chiral interest [15].

Chiral CE is highly regarded in chiral separation for its efficient separations, relatively short analysis times, rapid development of analytical methods, flexibility in selecting CSs, and low operational costs. This method requires only small amounts of solvents, analytes, and CSs, which also contributes to its minimal environmental impact, aligning it with the principles of "green chemistry" by consuming minimal amounts of organic solvents [15,16].

In chiral CE separations, two primary approaches can be employed: direct and indirect methods. Direct separations involve the use of CSs as additives to the background electrolyte (BGE), leading to the formation of diastereomeric associations between the CS and enantiomers, which are then separated based on differences in their mobility. Indirect separations, like those in HPLC, use a chiral derivatization reagent to form diastereomeric pairs that can be separated under achiral conditions. Chiral CE predominantly relies on direct separations; this focus on direct methods makes CE a highly effective and versatile technique for chiral analysis in pharmaceuticals [14,15].

A wide variety of CSs can be used in CE methods, including cyclodextrins (CD), macrocyclic antibiotics, crown ethers, polysaccharides, proteins, bile salts, and chiral micelles, among others [17]. Despite their differing mechanisms, these CSs facilitate separation through stereospecific interactions with chiral analytes. These interactions exhibit varying affinities towards enantiomers, leading to differences in their migration times under an electric field [18]. Among the available CSs, CD derivatives stand out as the most effective and widely used. Their popularity stems from their remarkable selectivity for a broad range of analytes, as well as other advantageous features such as UV transparency, commercial availability, and relatively good water solubility. These characteristics make CD derivatives well-suited for chiral analysis in CE methods [19,20].

Native CDs are cyclic oligosaccharides composed of glucopyranose units, forming structures with a truncated cone shape, consisting of 6 units (α-CD), 7 units (β-CD), or 8 units (γ-CD) of glucopyranose. The interior cavity of CDs is hydrophobic, facilitating hydrophobic interactions with analytes, while the exterior is hydrophilic, featuring primary and secondary hydroxyl groups exposed on the surface. CDs possess the ability to form inclusion complexes with a wide variety of substances due to their numerous chiral centers (β-CD, for example, contains 35 chiral centers). This structural feature allows for enantiorecognition both inside and/or outside the CD cavity [21,22].

CDs can be either native or derivatized, and they may be neutral or ionized (anionic or cationic). β-CD and its derivatives are the most widely used CDs in pharmaceutical analysis, primarily because their hydrophobic cavity is well-suited to include small to medium-sized molecules, which are typical of many medicinal substances. Most chiral pharmaceutical compounds contain aromatic systems, making them ideal candidates for inclusion within the β-CD cavity. This adaptability and effectiveness make β-CD and its derivatives a preferred choice in chiral separations using CE [21,23].

Chiral recognition can be enhanced by using ionizable, particularly anionic, CDs; by adjusting the pH of the BGE, the ionization of the CS can be optimized to achieve the desired chiral separation properties. In addition, the use of ionized CDs with an opposite charge to the analytes generates counter-current mobility, and this allows the use of lower concentrations of CSs [24].

In certain cases, the enantioseparation of some chiral analytes cannot be adequately achieved using a single CD as the CS. To enhance enantioresolution, a dual CD system is often employed, which typically involves combining a neutral (native or derivatized) CD with an ionized (often anionic) CD. This approach leverages the complementary interactions between the two types of CDs, improving the overall chiral separation effectiveness [25].

The use of CD derivatives in the form of a single isomer is advantageous compared to those with random substitutions because of higher reproducibility; however, pure CD prices can be, in many cases, prohibitive [26].

Chiral analysis of drugs marketed as pure enantiomers poses significant challenges due to the need to detect small amounts of enantiomeric impurities amidst a predominant enantiomer. According to the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) regulations, enantiomeric impurities must not exceed 0.1% of the main enantiomer. Achieving this goal requires high enantiomeric resolution to prevent the majority peak from overlapping with the impurity peak, as well as high sensitivity to detect the impurity at such low concentrations. An additional factor influencing the determination of enantiomeric impurities is the migration order of the enantiomers. Ideally, the enantiomeric impurity should migrate first, which helps to avoid any overlap of the main peak with that of the impurity [14,27].

The level of complexity in the optimization process of a chiral separation method to achieve adequate resolution is generally higher than in the case of an achiral separation. Traditionally, the optimization of analytical methods (HPLC, CE) is carried out using a univariate approach, the "One-Factor-at-Time" (OFAT) strategy; but this strategy does not allow the evaluation of interactions between experimental factors, which may lead to inadequate analytical method optimization and development. Over the past two decades, Design-of-Experiments (DoE) approaches have been frequently used to gain a better understanding of the multidimensional impacts and interactions of analytical parameters on analytical results [28,29].

This review aims to provide a comprehensive analysis of the application of CE techniques in evaluating the chiral purity of pharmaceuticals. By reviewing studies published over the past 15 years, this review aims to shed light on the advancements, methodologies, and trends within the field. The review also explores the development and optimization of CE methods, their role in ensuring the safety and efficacy of drugs, and how they meet regulatory requirements for chiral substances.

3. Review Methodology

An extensive literature search was conducted to identify relevant studies for inclusion in this review. Electronic databases, including Google Scholar, Scopus, and Web of Science, were searched using pertinent keywords such as "chiral purity", "enantiopurity", "capillary electrophoresis", and "chiral separation"/"enantioseparation". Boolean operators "and" and "or" were utilized to combine search terms effectively. Additionally, reference lists of relevant articles were manually screened to identify any additional studies that may have been missed during the initial search. Data synthesis was performed using a narrative approach, summarizing key findings and themes across the included studies.

4. Application of Capillary Electrophoresis for Enantiomeric Purity Analysis

A comprehensive selection of CE methods used for determining the chiral purity of pharmaceuticals was meticulously gathered and analyzed, covering research from the period 2010 to 2024. These articles were systematically organized based on the separation technique and discussed in a chronological manner to identify and elucidate the evolving

orientations and emerging tendencies in this field. By examining the progression and trends over these years, the review aims to provide a detailed understanding of how CE methodologies have developed and adapted to meet the growing and changing demands of chiral analysis in the pharmaceutical industry.

4.1. Capillary Zone Electrophoresis (CZE) Techniques

A stereoselective CZE assay was developed and validated by Wongwan and Scriba for determining the enantiomeric purity of *R*-chloroquine and *S*-chloroquine using sulfobutylether-β-CD (SBE-β-CD) as the CS. Chloroquine, a chemotherapeutic drug used for malaria prophylaxis and treatment, is used in therapy as a racemic mixture, but its enantiomers show differences in antimalarial activity and toxicity in vivo probably due to stereoselective metabolism. Separation was performed using a 100 mM phosphate BGE, at pH 2.5, containing 30 mg/mL SBE-β-CD. The CE method proved to be effective for both enantiomeric purity assessment and related impurity profiling of chloroquine, enabling the quantification of enantiomeric impurities down to 0.05%, revealing 0.24% of the enantiomeric impurity in a laboratory sample of *R*-chloroquine and detecting an unknown impurity at the 0.4% level [30].

A selective CZE method was developed by Liu et al. for determining the enantiomeric purity of RS86017, a potential antiarrhythmic agent with two chiral centers, using SBE-β-CD as the CS. The analytical parameters were optimized using the DoE strategy by orthogonal design, and the optimal conditions included 25 mM phosphate BGE at pH 8.0, containing 28 mg/mL SBE-β-CD and 20% acetonitrile (*v*/*v*). The method successfully separated the *R*,*S*-enantiomer of RS86017 from its potential chiral impurities (*S*,*R*-enantiomer, *R*,*R*-diastereomer, and *S*,*S*-diastereomer) within 10 min, demonstrating good resolution, linearity, and sensitivity (LODs and LOQs were 0.8 μg/mL and 2.5 μg/mL for all isomers). The method was validated and proved effective for assessing the chiral purity of RS86017 in bulk samples [31].

Deng et al. developed a CZE method for the enantioseparation of pramipexole, achieving optimal separation in under 6.5 min using a 50 mM phosphate BGE at pH 2.8 with 25 mM carboxymethyl-β-CD (CM-β-CD) as the CS. Pramipexole, an aminobenzothiazole derivative, is a dopamine receptor agonist used in early-stage Parkinson's disease, and it is used in the form of enantiopure *S*-pramipexole due to its higher affinity for dopamine receptors. Analytical parameters were optimized using an OFAT strategy. The method was applied to quantify pramipexole in commercially available immediate and sustained-release tablets. The developed method detected 0.3% of the *R*-pramipexole in bulk samples, making it suitable for enantiomeric purity testing [32].

Wang et al. developed a selective CZE method using SBE-β-CD for determining the enantiomeric impurity of *R*-modafinil (armodafinil). Modafinil is a wakefulness-promoting drug used in therapy as enantiopure armodafinil due to its longer half-life and higher bloodstream levels compared to *S*-modafinil. The optimized analytical conditions consisted of 20 mM phosphate BGE at pH 7.5, containing 20% methanol (*v*/*v*) and 20 mM SBE-β-CD as the CS; separation was achieved at 15 min. The method was successfully applied to assess the enantiomeric purity of armodafinil in bulk samples, proving effective for detecting *S*-modafinil as a chiral impurity [33].

Deng et al. developed a CZE method capable of quantifying 0.05% of *R*-citalopram and assaying the main component in *S*-citalopram (escitalopram) formulations. Citalopram is a selective serotonin reuptake inhibitor (SSRI) antidepressant, used in therapy both as a racemic mixture and in the form of pure enantiomer escitalopram, which shows higher efficacy and faster onset of action. Analytical parameters were optimized using an OFAT strategy. Optimal separation was achieved using a 25 mM phosphate BGE at pH 7.0 with 1.6% S-β-CD (*w*/*v*) as the CS; analysis was completed in a very fast time, less than 2 min. The method was applied to the quality control of escitalopram in bulk samples and tablets [34].

The same research group developed a chiral CZE to quantify 0.1% of the enantiomeric impurity, dextrocetirizine, in levocetirizine samples using S-β-CDs as the CS. Cetirizine is a second-generation H1 antihistaminic drug, used in therapy both as a racemic mixture and the pure enantiomer levocetirizine; the pharmacological effect resides mainly in the *R*-enantiomer. The optimal separation conditions were obtained using 50 mM borax BGE at pH 8.20, containing 1% S-β-CD (*w*/*v*). The method was successfully applied to the quality control of levocetirizine in bulk drugs and tablets, detecting 0.42% of dextrocetirizine in levocetirizine tablets [35].

A chiral CZE method was developed by Song et al. for the enantioseparation of *DL*-penicillamine across a nearly full pH range, using a one-pot labeling technique to introduce chromophore and charge groups onto the analytes. Penicillamine, a compound derived from penicillin, is used in the form of *D*-enantiomer for treating various conditions like Wilson disease, while the *L*-enantiomer is highly toxic and must be eliminated in *D*-penicillamine formulations. Utilizing neutral β-CD as a CS, baseline separation was achieved from pH 2.0 to over pH 10, with validation conducted at three pH levels: 4.5, 7.4, and 9.7. The LOD was 2.58 μg/mL in acidic and neutral BGEs and 1.41 μg/mL in basic BGEs. The method allowed for the quantification of enantiomeric *L*-penicillamine impurities down to 0.2%, 0.6%, and 2.0% for pH 4.5, 7.4, and 9.7, respectively. The method was successfully applied to the quantification of penicillamine enantiomers in commercial tablets and may be extendable to other chiral amines or amino acids [36].

A rapid and sensitive chiral CZE method was developed and validated by Deng et al. for determining levornidazole and its enantiomeric impurity dextrornidazole at a 0.05% level. Levornidazole is the *S*-enantiomer of ornidazole, a drug used for treating and preventing infections caused by anaerobic and microaerophilic protozoa and bacteria; it has similar clinical efficacy to racemic ornidazole but with fewer adverse effects. Analytical parameters were optimized using an OFAT strategy. Optimal separation conditions included a 20 mM Tris/phosphate BGE at pH 2.1 with 2.0% sulfated-α-CD (S-α-CD) (*w*/*v*) as the CS, achieving separation in less than 7 min. The method was successfully applied to the enantiomeric purity control of levornidazole in both bulk substance and injection solutions [37].

Lehnert et al. developed methods of separating the enantiomers of tolterodine and methoxytolterodine using S-α-CD, S-β-CD, and phosphated-γ-CD (P-γ-CD) as CSs by CZE in an acidic Tris/phosphate BGE at pH 2.5. Tolterodine is a medication used to alleviate urinary difficulties such as frequent urination and incontinence, while methoxytolterodine serves as a precursor in its synthesis; only *R*-tolterodine enantiomer possesses therapeutic properties. S-α-CD and S-β-CD allowed anodic detection, while P-γ-CD enabled cathodic detection; the study found that the use of P-γ-CD provided better results in terms of LOD, repeatability, and CS consumption. A reversal migration order of tolterodine and methoxytolterodine enantiomers was observed when S-α- and S-β-CD were used as the CSs. The methods were validated and successfully applied to determine 0.2% of *S*-tolterodine as an optical impurity in commercial tablets [38].

Qi and Zhang developed and validated a CZE method using CM-β-CD as a CS to determine the enantiomeric purity of levoamlodipine besylate bulk drugs. Amlodipine is a dihydropyridine calcium channel blocker used to treat hypertension and angina pectoris; it is administered as a racemic mixture; however, only *S*-amlodipine possesses vasodilation properties, while some side effects are associated with *R*-amlodipine. Optimal enantioresolution was achieved with a 40 mM phosphate BGE at pH 3.5 containing 4 mM CM-β-CD as the CS. The method was validated for determining the enantiomeric purity of *R*-amlodipine at the 0.2% level [39].

A chiral CZE method using S-β-CD as the CS was developed and validated by Zuo et al. for the enantioseparation of glycopyrrolate, an anticholinergic drug with two chiral centers, the *R*,*S*-enantiomer being the eutomer. Analytical parameters were optimized using an OFAT strategy. The optimal separation was achieved with a 30 mM phosphate BGE at pH 7.0 containing 2.0% S-β-CD (*w*/*v*), successfully separating *R*,*S*-glycopyrrolate from

its *S*,*R*-enantiomer, *R*,*R*-diastereomer, and *S*,*S*-diastereomer. The method demonstrated satisfactory resolution, linearity, and sensitivity, proving useful for the quality control and enantiomeric purity assessment of *R*,*S*-glycopyrrolate [40].

A CZE method using acetyl-β-CD (A-β-CD) as a CS was developed and validated by Lee et al. for determining the enantiomeric purity of valsartan. Valsartan is an angiotensin II receptor antagonist antihypertensive drug, used in therapy in the form of the pure enantiomer, *S*-valsartan. The optimized conditions included a 25 mM phosphate BGE at pH 8.0 with 10 mM A-β-CD as the CS. The method showed good linearity, accuracy, precision, and robustness, with an LOD of 0.01% and LOQ of 0.05%. The method was successfully applied to determine the chiral purity of valsartan tablets, identifying the *R*-enantiomer as a chiral impurity [41].

A CZE method using CDs was developed by Estevez et al. for the quality control of *S*-omeprazole (esomeprazole), regarding enantiomeric purity and related substances in raw materials and pellets. Omeprazole is a proton pump inhibitor (PPI) used in the treatment of gastric acid-related diseases; it is used in therapy both as a racemic mixture and as a pure enantiomer, esomeprazole, the latter exhibiting higher and more consistent bioavailability. Several parameters, including BGE and CD type and concentration, BGE additives concentration, and pH, were evaluated to calculate the resolution between omeprazole enantiomers and monitor the presence of related substances like omeprazole sulfone. Optimized conditions included a 100 mM Tris/phosphate BGE at pH 2.5 containing 20 mM 2-hydroxypropyl- β-CD (HP-β-CD) as the CS and 1 mM sodium dithionite. The method was validated, achieving LOD and LOQ for *R*-omeprazole of 0.6 μg/mL and 2.0 μg/mL, respectively, and could detect *R*-omeprazole at the USP limit of 0.2% [42].

Orlandini et al. developed a CZE method based on dual CD systems for the analysis of levosulpiride. Sulpiride is an antipsychotic drug belonging to the substituted benzamide class, functioning as a selective antagonist of central dopamine receptors; *S*-sulpiride is the biologically active form, exhibiting higher dopaminergic activity, as well as antiemetic and antidyspeptic effects, along with lower acute toxicity compared to both the *R*,*S*-sulpiride and *R*-sulpiride. This study is the first to apply the quality-by-design (QbD) framework to develop a CE system specifically for determining chiral impurities. The scouting phase enabled the selection of a suitable operative mode, which was CZE enhanced by a double CD system comprising negatively charged S-β-CD and neutral methylated-β-CD (M-β-CD). Key critical method attributes (CMAs) such as enantioresolution and analysis time were optimized using the dual CD system in reverse polarity mode. Design space for critical process parameters (CPPs) was defined via response surface methodology and Monte Carlo simulations. The method was used for the determination of levosulpiride and its chiral impurity in injection solutions [43].

Kazsoki et al. developed a CZE method for the enantioseparation of vildagliptin. Vildagliptin is an oral antidiabetic medication from the dipeptidyl-peptidase-4 (DPP-4) inhibitor class, used for treating type 2 diabetes in its enantiopure *S*-vildagliptin form. After screening 13 negatively charged CD derivatives as potential CSs, SBE-β-CD was selected for the enantioseparation. For the optimization of the analytical parameters, a factorial analysis study was conducted using an orthogonal experimental design. The optimized electrophoretic system required further refinement since the second peak (*S*-vildagliptin) migrated too close to the EOF, leading to potential inaccuracies in chiral impurity determination, which was addressed using an OFAT variation approach. The optimized method consisted of 75 mM acetate/Tris BGE at pH 4.75 containing 20 mM SBE-β-CD as the CS. LOD and LOQ values were found to be 2.5 and 7.5 μg/mL, respectively. The method is suitable for detecting the distomer at a 0.14% level. The method was used to determine the chiral purity of *S*-vildagliptin in commercial tablet formulations [44].

Ginterova et al. developed a CZE method to achieve chiral separation of cinacalcet. Cinacalcet, the first approved calcimimetic for treating secondary hyperparathyroidism in dialysis patients, is used in the form of enantiopure *R*-cinacalcet, which is more potent than *S*-cinacalcet. The optimum separation conditions consisted of 150 mM phosphate BGE at

pH 2.5, 0.5% hydroxypropyl-γ-CD (HP-γ-CD), and 20% methanol (*v*/*v*) as organic additive; enantiomers were separated within 12 min. The method demonstrated the ability to detect 0.1% *S*-cinacalcet in tablets. The method's precision and cost-effectiveness suggest it is a viable alternative to traditional HPLC techniques [45].

A QbD approach was used to develop a CZE method to evaluate the enantiomeric purity of *S*-ambrisentan by Krait et al. Ambrisentan is a diphenylpropanoic acid derivative, used to treat pulmonary arterial hypertension in the form of enantiopure *S*-ambrisentan. Initial CD screening identified γ-CD at pH 5.0 as a suitable CS, with further optimization using response surface methodology. This allowed for the determination of the design space, from which the experimental conditions for the optimal working point were established. The optimum separation conditions consisted of 50 mM sodium acetate BGE at pH 4.0 containing 30 mM γ-CD as the CS. The optimized method, validated according to ICH guidelines, allowed the determination of 0.1% *R*- ambrisentan in less than 10 min. The method was applied to a commercial sample, confirming its suitability for assessing the chiral purity of *S*-ambrisentan [46].

Another QbD approach was utilized by Niedermeier and Scriba to develop a CZE method for simultaneously determining dextromepromazine and levomepromazine sulfoxide impurities in levomepromazine. HP-γ-CD was selected as the optimum CS in the initial CD screening. The analytical target profile (ATP) was defined so that the method could quantify 0.1% of both impurities with a precision of ≤10%. CPPs like CD concentration, BGE concentration, pH, temperature, and voltage were optimized using fractional factorial resolution V+ DoE, followed by a central composite face-centered DoE used as response surface methodology for deriving the design space by Monte Carlo simulations. The optimized method used a 100 mM citric acid BGE at pH 2.85, with 3.6 mg/mL HP-γ-CD as the CS. The method, validated according to ICH guidelines, effectively determined impurities at the 0.1% level and was applied to both the European Pharmacopoeia (Eur Ph) reference substance and a commercial injection solution. Levomepromazine sulfoxide can theoretically be simultaneously analyzed using the CE method; however, the rapid oxidation of levomepromazine in aqueous solutions, even with antioxidants, complicates the precise determination of this impurity. Accurate data on the sulfoxide can only be obtained if the sample is analyzed immediately after preparation [47]. The developed CE method offers the first reliable approach to accurately determine the enantiomeric purity of levomepromazine, effectively limiting dextromepromazine content to 0.1%, overcoming the inaccuracies of current pharmacopeial methods (due to the low specific optical rotation of levomepromazine).

Szabó et al. investigated the chiral recognition of lenalidomide using CDs and polysaccharides as CSs. Lenalidomide is a thalidomide analogue with immunomodulatory, antiangiogenic, and antineoplastic properties; even though the *S*-enantiomer reported higher potency, the development of single enantiomer formulations was halted due to rapid in vivo racemization. HPLC with polysaccharide-type chiral stationary phases (CSP) achieved enantioseparation in a polar organic mode. In CZE, after an extensive CD screening, SBE-β-CD was selected as the optimal CS; where quasi-equal stability constants indicated that enantiodiscrimination was primarily driven by the different mobilities of the transient diastereomeric complexes. Validated methods for determining 0.1% *R*-lenalidomide as a chiral impurity were developed using both optimized HPLC (Chiralcel OJ column, pure ethanol, 0.6 mL/min flow rate) and CE (30 mM phosphate BGE, pH 6.5, 30 mM SBE-β-CD) [48]. The results can be important for ensuring chiral purity in lenalidomide production, especially if a racemic switch development is implemented.

Meng and Kang developed and validated a CE method to detect stereoisomeric impurities in sitafloxacin. Sitafloxacin, a fluoroquinolone derivative, possesses three chiral centers, leading to the potential formation of eight stereoisomers. Depending on the synthetic pathway used, the bulk substance may contain at least four of these stereoisomers; some of these stereoisomers exhibit activity against human type II topoisomerase, which could be linked to their cytotoxic effects. The method uses a dual CS system composed

of γ-CD and a Cu^{2+}-*D*-phenylalanine complex, achieving high-resolution separation of both enantiomers and diastereoisomers. CPPs such as γ-CD, Cu^{2+}, and *D*-phenylalanine concentrations, along with pH, were optimized using response surface methodology with a face-centered central composite DoE. The final optimized conditions included 15 mM phosphate BGE at pH 4.5 with 15 mM *D*-phenylalanine, 20 mM $CuSO_4$, and 20 mM β-CD. This method, validated and robust, can detect stereoisomeric impurities of sitafloxacin as low as 0.1% [49].

The enantiomers of praziquantel, an anthelmintic drug commonly used for treating schistosomiasis, were successfully separated by Szabó et al. using CZE with CDs as the CS. Praziquantel has a pyrazino-isoquinoline structure and is marketed as a racemate; *R*-praziquantel provides the antischistosomal activity, while the inactive *S*-praziquantel increases tablet size and cytotoxicity and contributes to the drug's unpleasant taste. Nine different anionic CDs were tested to evaluate their ability to differentiate between praziquantel enantiomers, which are uncharged, and seven CDs demonstrated chiral interactions with the enantiomers; S-β-CD was found to be the most effective CS. S-β-CD showed nearly equal stability constants for the enantiomers but had high selectivity due to the different mobilities of the transient diastereomeric complexes. Initially, the enantiomer migration order was unfavorable; however, switching the polarity (detection end at the anode) reversed the migration order and achieved an exceptionally high resolution (Rs ≈ 35), albeit with longer migration times (Figure 1). The optimized separation conditions included 50 mM phosphate BGE at pH 2.0 and 15 mM S-β-CD as the CS, and the analysis time was under 10 min. The method was validated in accordance with ICH guidelines and tested on in-house synthesized batches of *R*-praziquantel, as well as on commercial combination tablets containing the racemic mixture of praziquantel [50].

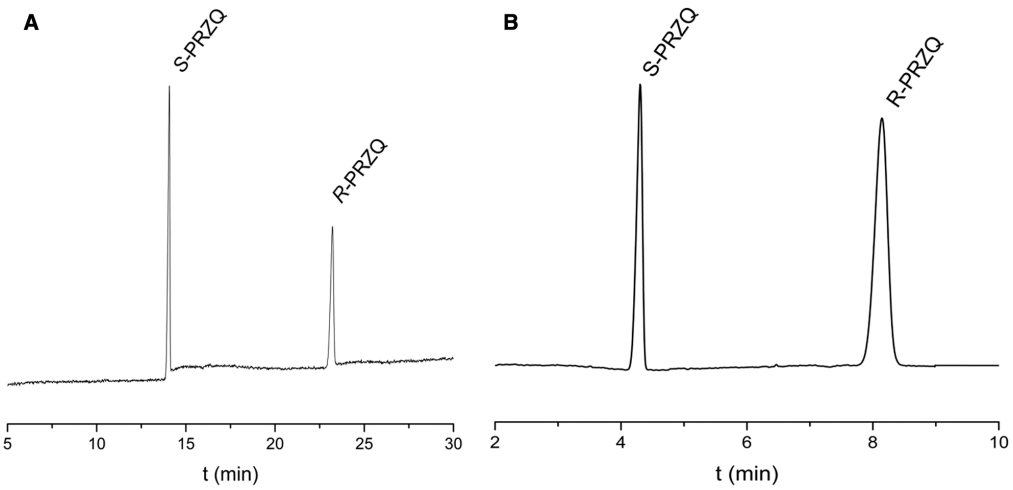

Figure 1. Chiral separation of praziquantel (PRZQ)-enantiomer migration order reversal by polarity switching: (**A**) long-end injection (*S*-PRZQ vs. *R*-PRZQ), (**B**) short-end injection (*R*-PRZQ vs. *S*-PRZQ) (electrophoretic conditions: 50 mM phosphate, pH 2.5, 15 mM S-β-CD, (**A**) 15 kV to the anode, (**B**) 15 kV to the cathode, 25 °C, 50 mbar × 2 s, UV detection 210 nm), reprinted from Szabó [50] with permission from Wiley.

A CZE method for determining the enantiomeric purity of pregabalin after derivatization with dansyl hydrochloride was developed using DoE by Harnisch et al. Pregabalin is a γ-aminobutyric acid analog used in therapy in the form of enantiopure *S*-pregabalin. A D-optimal DoE identified CPP, while a central composite face-centered DoE and Monte Carlo simulations defined the method's design space. The optimized conditions included a 100 mM phosphate BGE at pH 2.5, containing 40 mg/mL heptakis(2,3,6-tri-O-methyl)-β-CD (HTM-β-CD). The method's robustness was confirmed using a Plackett–Burman DoE and validated according to the ICH guideline Q2(R1). The method can detect *R*-pregabalin at the 0.015% level and had an LOQ at 0.05%, relative to a 1.59 mg/mL pregabalin sam-

ple. The LOD for *R*-pregabalin was 0.015%, significantly lower than the pharmacopeial limit of 0.15%. This method demonstrated good precision and accuracy when applied to commercial capsules, where the *R*-pregabalin content was below the LOD [51].

A CZE method was developed by Pasquini et al. for determining the enantiomeric purity and chemically related impurities of cinacalcet, following QbD principles. After an initial CD screening, in which carboxyethyl-β-CD (CE-β-CD) and HP-γ-CD showed chiral interactions with the analyte, HP-γ-CD was chosen as the optimum CS. CPPs like pH, HP-γ-CD, and methanol concentration and voltage were optimized using a multivariate approach. A Box–Behnken DoE facilitated the creation of contour plots and highlighted quadratic and interaction effects, leading to the identification of the method operable design region (MODR) through Monte Carlo simulations. The optimized conditions included 150 mM phosphate BGE at pH 2.7, 3.1 mM HP-γ-CD, and 2% methanol (*v*/*v*). The method achieved complete analyte separation in approximately 10 min and was validated according to ICH guidelines. This systematic QbD approach not only facilitated method development but also enhanced understanding and risk management. The validated method was successfully applied to analyze cinacalcet tablets, demonstrating its efficacy in controlling enantiomeric purity and profiling impurities [52].

A CZE method was developed by Krait et al. for detecting levomethorphan as an impurity in dextromethorphan formulations, targeting a precision and accuracy level of 0.1%. Dextromethorphan is a centrally acting antitussive medication, while its enantiomer, levomethorphan, is an illicit substance known for its opioid analgesic properties. The study utilized a dual-selector system with S-β-CD and methyl-α-cyclodextrin (M-α-CD) to achieve optimal separation. The inclusion of M-α-CD alongside S-β-CD significantly improved peak shapes by reducing the overall mobility of the two enantiomers despite a slight reduction in enantioresolution. Initial scouting experiments and a fractional factorial resolution IV DoE identified the CPPs. These were further refined using a central composite, face-centered DoE and Monte Carlo simulations to establish the method design space. The optimized method employed 30 mM phosphate BGE at pH 6.5, 16 mg/mL S-β-CD, and 14 mg/mL M-α-CD. Validation followed the ICH guideline Q2(R1), and the method was applied to analyzing capsule formulations of dextromethorphan. The apparent binding constants and complex mobilities of the enantiomers with the CDs were determined, shedding light on their migration behavior [53].

Krait and Scriba developed a CZE method for the determination of the enantiomeric purity of dexmedetomidine allowing the quantitation of levomedetomidine at the 0.1% level. Dexmedetomidine is a selective α2-adrenergic agonist used as a general anesthetic, whereas its enantiomer, levomedetomidine, does not possess sedative effects. Initial CD screening experiments identified S-β-CD as the optimal CD. The CPPs were determined using a fractional factorial resolution V+ DoE, followed by a central composite face-centered DoE and Monte Carlo simulations to define the design space. The optimized conditions included 50 mM sodium phosphate BGE at pH 6.5 containing 40 mg/mL S-β-CD as the CS; analysis time was under 10 min [54]. Interestingly, no levomedetomidine was detected in commercial batches of dexmedetomidine, suggesting the USP 1.0% limit may be overly high, potentially due to differences in chromatographic assay techniques. Figure 2A displays an electropherogram of standards under optimized experimental conditions, while Figure 2B presents a representative electropherogram of dexmedetomidine spiked with levomedetomidine at the LOQ (0.1% level); Figure 2C shows an electropherogram from a pharmaceutical formulation.

Fayed et al. developed a CZE method for separating clopidogrel bisulphate and three of its impurities (*R*-clopidogrel, *R*,*S*-clopidogrel, and a hydrolysis product). Clopidogrel is a dihydrothienopyridine pro-drug that irreversibly inhibits platelet ADP receptors, providing potent anti-platelet and anti-thrombotic effects; it is used in therapy in the form of enantiopure *S*-clopidogrel, the distomer *R*-clopidogrel being inactive. The optimization and robustness of the method were studied using a reduced central composite DoE. The optimal separation conditions were achieved with a 10 mM triethylamine–phosphoric

acid BGE at pH 2.3, containing 5% S-γ-CD; the separation was achieved in 17 min. The method was validated for quantitative determination of clopidogrel, demonstrating a limit of detection LOD of 0.13 μg/mL and an LOQ of 0.4 μg/mL. The CZE method was successfully applied to analyze commercial bulk samples of clopidogrel, confirming its suitability for quality control purposes [55].

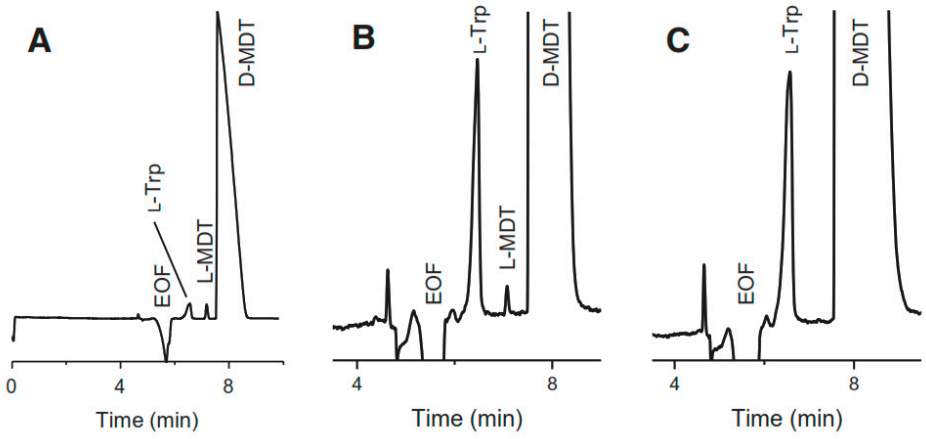

Figure 2. Electropherograms of a solution containing 2 mg/mL dexmedetomidine (*D*-MDT) spiked with (**A**) 20 μg/mL levomedetomidine (*L*-MDT) (1.0% relative concentration) and (**B**) 2 μg/mL levomedetomidine (0.1% relative concentration). (**C**) Analysis of a commercial dexmedetomidine hydrochloride sample at a concentration equivalent to 2 mg/mL dexmedetomidine (electrophoretic conditions: 50 mM phosphate, pH 6.5, 40 mg/mL S-β-CD, 10 kV, 17 °C, UV detection at 200 nm). Reprinted from Krait and Scriba [54] with permission from Wiley.

A CZE method was developed and validated by Harnisch and Scriba for determining the purity of *S*-dapoxetine, focusing on three chemical related substances and the distomer *R*-dapoxetine. Dapoxetine, a short-acting SSRI, was initially developed for depression but is now used to treat premature ejaculation due to its rapid onset and elimination profile; it is used in therapy in the form of enantiopure *S*-dapoxetine. The method utilized a dual CD system optimized using a fractional factorial resolution V+ DoE, followed by a central composite face-centered DoE and Monte Carlo simulations to define the design space. The optimal separation conditions consisted of 50 mM phosphate BGE at pH 6.3, containing 45 mg/mL S-γ-CD and 40.2 mg/mL 2,6-dimethyl-β-CD (DM-β-CD). Robustness testing using a Plackett–Burman DoE and validation according to the ICH guideline Q2(R1) demonstrated the method's effectiveness in the 0.05–1.0% range relative to *S*-dapoxetine concentration. The method was successfully applied to both drug substances and commercial tablets, showing that the chemically related substances (3*S*)-3-amino-3-phenylpropan-1-ol, (3*S*)-3-(dimethylamino)-3-phenylpropan-1-ol, and 1-naphthol were not present above the LOD. The CE method also detected an additional impurity, expanding the spectrum of detectable compounds beyond those initially considered. For enantiomeric purity, the CE assay proved comparable to the previously developed HPLC method, identifying a 70:30% scalemic mixture in a sample sold as a racemate and detecting the *R*-dapoxetine enantiomer in one sample at levels between 3.6% and 3.9%. Figure 3A presents an electropherogram of a standard solution containing all the studied compounds, while Figure 3B shows an electropherogram of a sample with all impurities at the 0.05% level; electropherograms of a dapoxetine hydrochloride standard sample and tablets are displayed in Figure 3C,D, respectively [56].

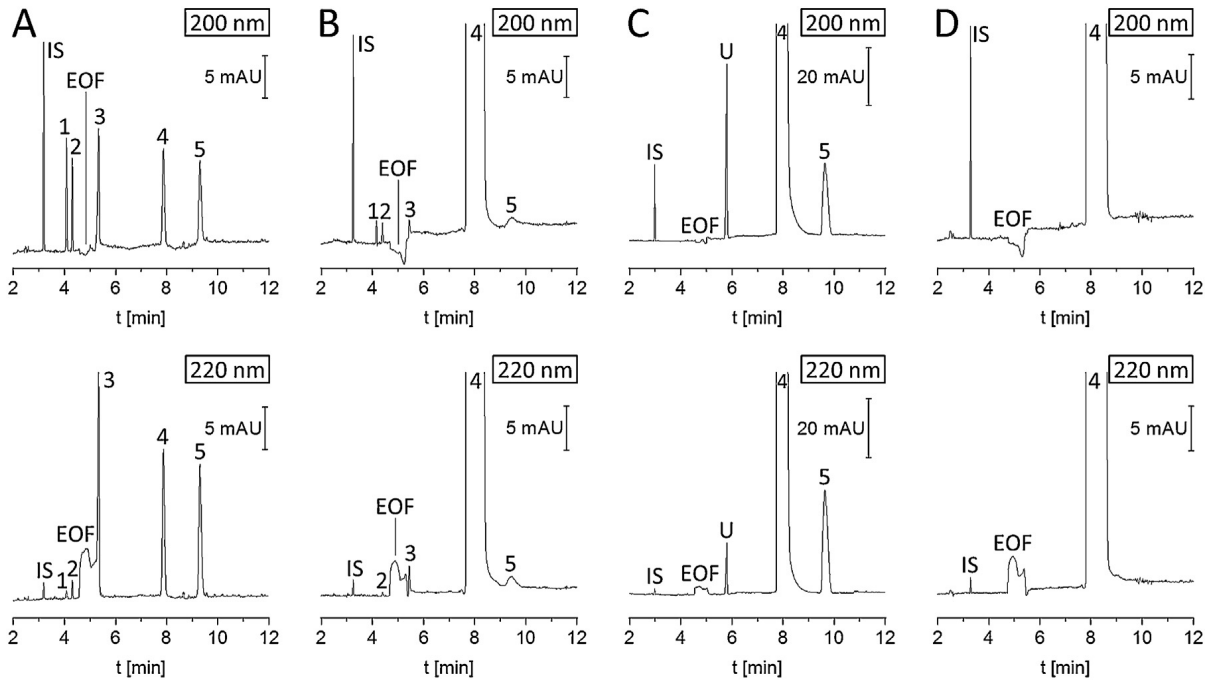

Figure 3. Electropherograms of (**A**) standard solution containing 12.5 µg/mL of all test compounds, (**B**) solution of 5 mg/mL *S*-dapoxetine spiked with all test compounds at 0.05%level, (**C**) solution of 5 mg/mL *S*-dapoxetine, and (**D**) solution of Priligy® tablets equivalent to 5 mg/mL *S*-dapoxetine. Peak labelling: 1—3*S*-3-amino-3-phenylpropan-1-ol; 2—3*S*-3-(dimethylamino)-3-phenylpropan-1-ol; 3—1-naphthol; 4—*S*-(+)-N,N-dimethyl-1-phenyl-3-(1-naphthalenyloxy)propanamine–*S*-dapoxetine; 5—*R*-dapoxetine; IS—4-aminopyridine (12 µg/mL); U—unknown impurity. (Electrophoretic conditions: 50 mM sodium phosphate, pH 6.3, 45 mg/mL S-β-CD, 40.2 mg/mL DM-β-CD, 9 kV, 15 °C, UV detection at 200 and 220 nm). Reprinted from Harnisch and Scriba [56] with permission from Wiley.

Comparing the article by Harnisch and Scriba [56] with an earlier work by Neumajer et al. [57], we observe that Harnisch and Scriba explored a CE method targeting the determination of *R*-dapoxetine and its related impurities, utilizing a dual CD system optimized via DoE and validated for pharmaceutical applications, while Neumajer et al. focused on developing a validated CE method that employs M-γ-CD as the CS, with an emphasis on method optimization through orthogonal experimental design to achieve enhanced resolution.

Two distinct CZE methods using CDs as CSs were developed and validated for the enantiomeric separation of two PPI derivatives, lansoprazole and rabeprazole. Lansoprazole and rabeprazole are used primarily as racemic mixtures, but their enantiopure forms (dexlansoprazole and dexrabeprazole) offer superior metabolic and pharmacokinetic profiles. Fourteen different neutral and anionic CDs were initially screened at pH 4 and 7; SBE-β-CD with a degree of substitution (DS) of 6.5 and 10 at neutral pH emerged as the most effective CS for both compounds. Further comparisons of various dual CD systems identified a combination of SBE-β-CD (DS 6.5) and native γ-CD as the most suitable for the separations. Method optimization followed a multivariate approach, beginning with a fractional factorial DoE for screening and then employing a central composite DoE to fine-tune analytical conditions. The optimized methods for lansoprazole and rabeprazole involved using a 25 mM phosphate BGE at pH 7, with specific concentrations of the CDs (10 mM SBE-β-CD, 20 mM γ-CD for lansoprazole; 15 mM SBE-β-CD, 30 mM γ-CD for rabeprazole). Both methods ensured the *S*-enantiomers migrated first and were validated according to current ICH guidelines, proving reliable, linear, precise, and accurate for determining 0.15% distomer as a chiral impurity in dexlansoprazole and dexrabeprazole samples. Representa-

tive electropherograms with optimal parameters, illustrating 0.15% chiral impurities, are shown in Figure 4A for dexlansoprazole and in Figure 4B for dexrabeprazole [58].

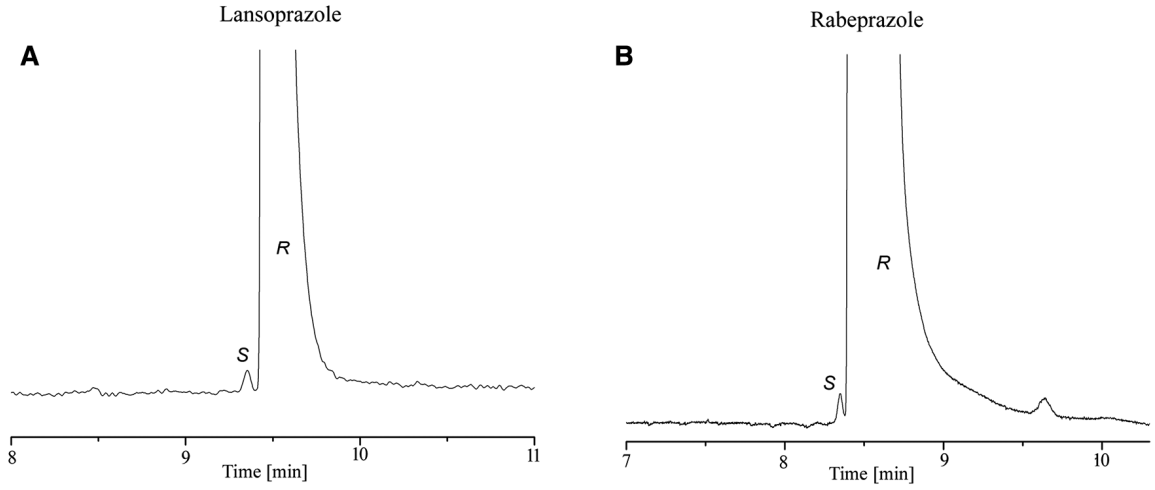

Figure 4. Determination of *S*-lansoprazole and *S*-rabeprazole in *R*-lansoprazole (**A**) and R-rabeprazole (**B**) samples, each containing 0.15% chiral impurities (electrophoretic conditions: 25 mM phosphate, pH-7.0, 10 mM SBE-β-CD/20 mM γ-CD, 20 kV, 17 °C, UV detection 210 nm–lansoprazole; 25 mM phosphate, pH-7.0, 15 mM SBE-β-CD/30 mM γ-CD, 20 kV; 18 °C, UV detection 210 nm–rabeprazole). Reprinted from Papp et al. [58] with permission from Wiley.

A stereospecific CE method was developed and validated by Szabó et al. for determining the enantiomeric purity of *R*-rasagiline. Rasagiline is an irreversible inhibitor of monoamine oxidase B used to treat Parkinson's disease symptoms and is marketed as a pure enantiomer *R*-rasagiline. The optimized method utilized a 50 mM glycine–chlorohydric acid BGE at pH 2.0, containing 30 mM SBE-β-CD as the CS; the analysis time was under 8 min. The method was validated according to ICH guidelines, proving to be reliable, linear, precise, and accurate for detecting 0.15% *S*-rasagiline as a chiral impurity in *R*-rasagiline samples and quantifying the eutomer, being successfully applied to a commercial tablet formulation. To further understand the enantiomeric separation, 1H and 2D NOESY were used to determine the spatial structure of diastereomeric associates, revealing that the aromatic moiety of rasagiline can enter the CD cavity. Nuclear magnetic resonance (NMR) titration and molecular modeling showed that *S*-rasagiline forms a more stable inclusion complex with SBE-β-CD compared to *R*-rasagiline, in concordance with the electrophoretic results [59]. The combination of CE with NMR spectroscopy and molecular modeling offered a deeper insight into the chiral recognition mechanism.

A CZE method was developed and validated by Mai et al. to determine the enantiomeric impurity of linagliptin. Linagliptin is a DPP-4 inhibitor that is used orally in the treatment of type 2 diabetes in the form of enantiopure *R*-linagliptin. The analytical optimization was guided by a DoE approach, focusing on CPPs such as BGE concentration, pH, CD concentration, temperature, and voltage. The optimized conditions comprised 70 mM sodium acetate BGE at pH 6.10 and 4.7 mM CM-β-CD as the CS; chiral resolution was achieved in 10 min. The method demonstrated an LOQ of 0.05% for the impurity and was validated for quality control of linagliptin bulk drug [60].

A CZE method was developed by Niedermeier and Scriba using a QbD approach for the simultaneous determination of dextrodropropizine and the achiral precursor 1-phenylpiperazine in levodropropizine. Levodropropizine is a non-opioid, peripheral antitussive medication used for the short-term relief of cough. The method aimed to detect these impurities below the 0.5% level within a maximum analysis time of 20 min, ensuring precision and accuracy. Initial CD screening experiments identified S-β-CD as the suitable CS. CPPs, including CD concentration, propan-2-ol concentration, temperature,

and voltage, were optimized using a full factorial DoE followed by a central composite face-centered DoE; Monte Carlo simulation was used to define the design space. The optimized method consisted of a 25 mM phosphate BGE at pH 7.0 containing 23.5 mg/mL S-β-CD and 10% propan-2-ol (*v*/*v*). Robustness was evaluated using a Plackett–Burman DoE, and the method was validated according to ICH guidelines. The method was successfully applied to analyze levodropropizine reference substance and a liquid dosage form. Although it was less sensitive and required a longer analysis time compared to a previously published HPLC method, the CE method performances still met the Eur. Ph. requirements for LOD and LOQ [61].

A CZE method was developed and validated by Krait et al. for assessing the chiral purity of tenofovir, applying a QbD approach. Tenofovir is a nucleotide analogue reverse transcriptase inhibitor effective against retroviruses such as HIV-1, HIV-2, and hepadnaviruses and is used therapeutically in the form of enantiopure *R*-tenofovir. Through an initial CD screening, quaternary ammonium β-CD(QA-β-CD) was identified as the optimal CS. Method optimization was achieved using a fractional factorial resolution V+ DoE followed by a central composite face-centered DoE. The optimized method involved 100 mM phosphate BGE at pH 6.4 containing 45 mg/mL QA-β-CD. Robustness was confirmed via a Plackett–Burman DoE and the method was validated according to the ICH Q2(R1) guidelines, enabling the determination of the *S*-tenofovir at the 0.1% level [62]. This study exemplifies the effectiveness of CE for chiral purity assessments, highlighting the importance of meticulous experimental control even for robust methods.

A stereospecific CZE method was developed by Szabó et al. to separate the enantiomers of apremilast. Apremilast is an orally active drug used to treat psoriasis and psoriatic arthritis by inhibiting phosphodiesterase 4; it is used in therapy in the form of enantiopure *S*-apremilast. Six anionic CD derivatives were tested for their ability to discriminate between the enantiomers; only succinyl-β-CD (Succ-β-CD) demonstrated chiral interactions, but the enantiomer migration order was unfavorable, with the eutomer migrating faster. Despite optimizing CPPs such as pH, CD concentration, CD degree of substitution, and temperature, the method was inadequate for purity control due to low resolution and the unfavorable migration order. To address this, the EOF direction was altered by dynamically coating the capillary's inner surface with poly(diallyldimethylammonium) or polybrene, resulting in migration order reversal. The dynamic capillary coating offers a general solution for migration order reversal, especially when the CS is a weak acid [63]. Obtaining the optimal migration order is essential for assessing the enantiomeric purity of chiral drugs, as commonly used CSs in chromatography typically do not permit for intentional reversal of enantiomer elution sequences. This approach underscores the simplicity and adaptability of developing enantioselective CE methods.

Papp et al. developed a CZE method for the determination of enantiomeric purity of vildagliptin. Vildagliptin is a DPP-4 inhibitor used for treating type 2 diabetes in the form of enantiopure *S*-vildagliptin. An initial CD screening was conducted to screen 16 CD derivatives for their effectiveness as CSs at different pH levels using phosphate and acetate BGE. Method optimization was achieved through a DoE approach, considering variables such as BGE and CD concentration, pH, temperature, and voltage to improve chiral resolution and analysis time, first performing screening for the identification of significant parameters by fractional factorial DoE, followed by a face-centered central composite optimization DoE. The optimal conditions identified were a 75 mM acetate BGE at pH 4.5 with 50 mM α-CD as the CS, achieving baseline enantioseparation of vildagliptin within 9 min. The method's analytical performance was evaluated and applied to assess the enantiomeric purity of *S*-vildagliptin in pharmaceutical formulations. NMR measurements and molecular modeling revealed that α-CD forms a 1:1 inclusion complex with vildagliptin, with *S*-vildagliptin showing stronger binding consistent with the observed migration order [64].

The article published previously by Kazsoki et al. [44] is centered on the development and validation of a CD-modified CE method, with a strong emphasis on optimizing experi-

mental conditions like temperature and voltage for practical applications in enantiomeric impurity control of vildagliptin, while Papp et al. [64] focus on the chiral separation using CE, emphasizing the study of enantiomeric complexation through NMR and molecular modeling to gain insights into the chiral recognition process.

Diquats, derivatives of the herbicide diquat, are emerging as significant functional organic molecules with potential applications in supramolecular chemistry, chiral catalysis, and chiral analysis. For these applications, enantiomerically pure forms of diquats are required, necessitating the development of a method to separate and verify the purity of *P*- and *M*-isomers. A chiral CE method has been developed by Bilek et al. for this purpose, achieving fast and baseline separation of enantiomers of 11 new diquats within 5–7 min. This was accomplished using a 22 mM sodium hydroxide, 35 mM phosphoric acid BGE at pH 2.5, and 6 mM randomly sulfated α-, β-, and γ-CDs as CSs. S-γ-CD proved to be the most effective CS baseline separating all 11 diquat enantiomers, followed by S-β-CD and S-α-CD, which separated 10 and 9 enantiomers, respectively. The method confirmed high enantiopurity for the isolated *P*- and *M*-enantiomers of three diquats and identified their migration order. The method was used as a control analytical method for determining enantiopurity in newly prepared diquat enantiomers, enhancing the preparative resolution process [65].

A CZE method was developed by Modroiu et al. for controlling the enantiomeric purity of silodisin using a QbD strategy. Silodosin is a selective α1-adrenoreceptor antagonist used in treating benign prostatic hyperplasia; it is used in therapy in the form of enantiopure *R*-silodosin. After a complex CD screening of neutral and ionized CDs at different pH levels, CM-β-CD was selected as the optimum CS. Initial scouting led to a two-level full factorial DoE to identify CPPs. Subsequent optimization using a face-centered central composite DoE established the final separation conditions, which included the use of 100 mM phosphate BGE at pH 2.9 and 40 mg/mL CM-β-CD. Robustness testing with a Plackett–Burman DoE emphasized the necessity of precise pH control to ensure optimal peak shape and resolution. The method was validated according to the ICH guideline Q2 (R1), demonstrating its applicability to both drug substances and pharmaceutical formulations. Monte Carlo simulations were employed to define the method's design space, ensuring reliable detection of the enantiomeric impurity *S*-SLD at concentrations as low as 0.02%. Figure 5 displays the electropherogram obtained under optimized experimental conditions, showing the presence of *S*-silodosin at different concentration levels relative to *R*-silodosin [66].

The same research group developed a CZE method to determine the enantiomeric purity and chemically related impurities of tamsulosin using QbD principles and DoE. Tamsulosin is an α1-adrenoceptor antagonist used for treating lower urinary tract symptoms associated with benign prostatic hyperplasia, with *R*-tamsulosin being used in therapy as a pure enantiomer. A dual CD system consisting of S-β-CD and carboxymethyl-α-CD (CM-α-CD) was selected as the CS after initial CD scouting experiments. CPPs were identified using a fractional factorial resolution V+ DoE, followed by optimization with a face-centered central composite DoE and Monte Carlo simulations to define the method's design space. The optimized experimental conditions were 30 mM phosphate BGE at pH 3.0, containing 40 mg/mL S-β-CD and 7 mg/mL CM-α-CD. Robustness was assessed using a Plackett–Burman DoE, and the method was validated according to ICH Q2(R1) guidelines. The validated method allowed for the quantification of the chiral impurity *S*-tamsulosin and three other related impurities at the 0.1% level with acceptable accuracy and precision, though one chemical-related impurity showed a lack of precision during validation. Figure 6 displays an electropherogram obtained under optimized experimental conditions, showing the presence of chiral and achiral impurities of tamsulosin at a 0.1% concentration level relative to *R*-tamsulosin [67].

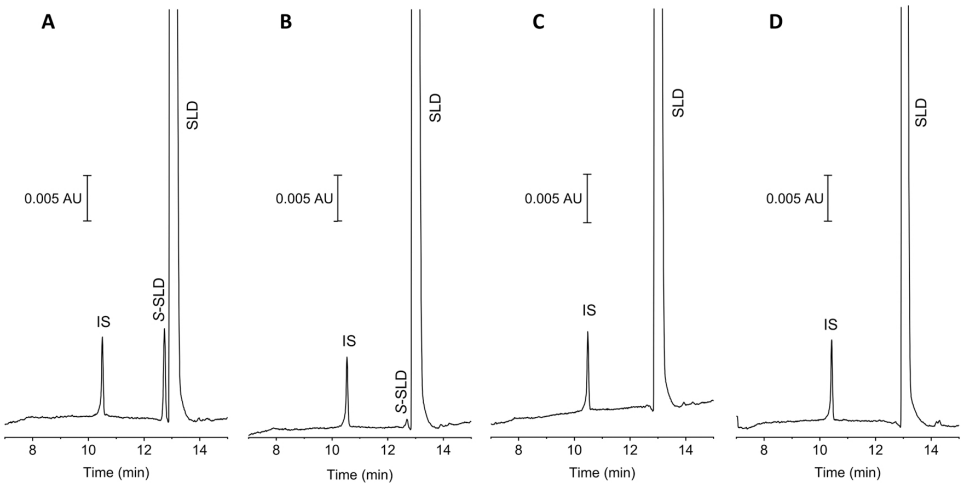

Figure 5. Electropherograms of samples containing 2 mg/mL *R*-silodosin (SLD) and 30 µg/mL lidocaine hydrochloride (IS), spiked with (**A**) 20 µg/mL *S*-silodosin (*S*-SLD) (1% relative concentration), (**B**) 1.4 µg/mL *S*-silodosin (0.07% relative concentration), (**C**) unspiked sample, and (**D**) sample obtained from Urorec® capsules (electrophoretic conditions: 100 mM phosphate, pH 2.9, 40 mg/mL CM-β-CD, 28 kV, 17 °C, UV detection at 200 nm). Reprinted from Modroiu et al. [66] with permission from Elsevier.

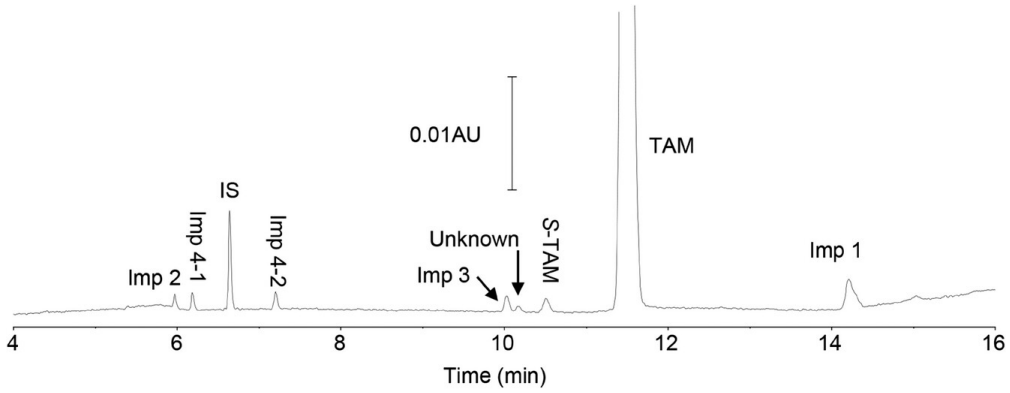

Figure 6. Representative electropherogram of a sample containing 2 mg/mL *R*-tamsulosin (TAM) and 20 µg/mL silodosin (IS), spiked with 2 µg/mL *S*-tamsulosin (*S*-TAM), 2 µg/mL each of chemical-related impurities 1, 2, and 3, and 4 µg/mL of impurity 4 (0.1% relative concentrations) (electrophoretic conditions: 30 mM sodium phosphate, pH 3.0, 40 mg/mL S-β-CD, 7 mg/mL CM-α-CD, −23 kV, 18 °C, UV detection 200 nm). Imp 4-1 and Imp 4-2 represent the enantiomers of Imp 4. Reprinted from Modroiu et al. [67] with permission from Wiley.

4.2. Other CE Techniques

Borst and Holzgrabe developed a chiral microemulsion electrokinetic chromatography (MEEKC) method for the enantiomeric separation of several phenethylamine derivatives (diethylnorephedrine, epinephrine, ephedrine, N-methylephedrine, norephedrine, pseudoephedrine, 2-amino-1-phenylethanol, and 2-(dibutylamino)-1-phenyl-1-propanol), employing an oil-in-water microemulsion, composed of ethyl acetate, sodium dodecyl sulfate (SDS), 1-butanol, propan-2-ol, and a 20 mM phosphate BGE at pH 2.5, with sulfated-β-CD (S-β-CD) as the CS. The MEEKC method, compared to a CZE method using heptakis(2,3-di-O-diacetyl-6-O-sulfo)-β-CD (HDAS- β-CD), proved to be cost-effective and versatile for impurity analysis in pharmaceutical analysis, though it required multiple runs for complete separation and had higher limits of detection (LOD) [68].

Rousseau et al. verified the enantiomeric purity of a synthetic intermediate of 3,4-dihydro-2,2-dimethyl-2H-1-benzopyran derivatives; 4-amino-2,2-dimethyl-6-

ethoxycarbonylamino-3,4-dihydro-2H-1-benzopyran was determined using non-aqueous capillary electrophoresis (NACE) and a combination of an anionic CD derivative and a chiral ionic liquid (CIL). The optimized NACE method employed 0.75 M formic acid, 10 mM ammonium formate as BGE, 5 mM ethylcholine bis(trifluoromethylsulfonyl)imide as CIL, and 1.5 mM heptakis(2,3-di-O-methyl-6-O-sulfo)-β-CD (HDMS- β-CD) as the CS, allowed the detection of 0.1% enantiomeric impurities, and was validated for its selectivity, precision, accuracy, and linearity [69].

Two chiral CE methodologies with UV and mass spectrometry (MS) detection were developed by Sánchez-López et al. for quality control of duloxetine. Duloxetine is a serotonin and norepinephrine reuptake inhibitor (SNRI) used in therapy in the form of enantiopure *S*-duloxetine, which is more potent than its distomer. Both methods achieved high baseline enantioresolution and good precision. The CE-MS method significantly improved sensitivity (LOD—20 ng/mL) compared to CE-UV (LOD—200 ng/mL), detecting duloxetine enantiomeric impurity at 0.02%. Validated and applied to four pharmaceutical formulations, both methods showed consistent results with labeled content and no detectable *R*-duloxetine impurity. Adjustments for CE-MS included increasing capillary length, resulting in longer migration times [70].

A comprehensive study was conducted by Pasquini et al. on the CE separation mechanisms and inclusion complexation with CD of *S*-ambrisentan, its distomer *R*-ambrisentan, and other impurities. A MEKC method was previously developed to simultaneously determine the enantiomeric purity and chemical impurities of *S*-ambrisentan using SDS micelles and γ-CD in a borate BGE. This study examined the electrophoretic behavior of the analytes with various CDs, highlighting γ-CD capacity to separate all studied compounds. A QbD approach was meticulously applied during method development, with a design space established through a risk-of-failure map and a selected probability level. The best results were obtained when using 100 mM borate BGE at pH 9.2, 100 mM SDS, and 50 mM γ-CD as the CS [71]. Molecular modeling simulated aggregates between SDS micelles and analytes and inclusion complexes between CD, SDS, and analytes. The energy calculations of these complexes indicated a γ-CD strong tendency to form mixed complexes with SDS and analytes. Two-dimensional Nuclear Overhauser effect spectroscopy (NOESY) confirmed these interactions, particularly between the analytes' aromatic moiety, the SDS aliphatic chain, and γ-CD protons. The research concluded that the separation mechanism in the CD-MEKC method involves significant interactions between the analytes and CDs, with γ-CD and SDS playing crucial roles [72].

While the article published previously by Krait et al. focuses on a QbD approach to developing a CE method for determining the chiral purity of ambrisentan, emphasizing method optimization and robustness [46], the method by Pasquini et al. explores a combined approach using CE, NMR, and molecular modeling to investigate the intermolecular interactions and separation mechanisms of ambrisentan and its related impurities, offering a more detailed molecular-level understanding of the separation process [72].

Ma et al. developed an innovative CIL based on SBE-β-CD for enantioseparation in CE, demonstrating superior enantioselectivity compared to the classic approach using SBE-β-CD. The study optimized factors such as CIL concentration, organic solvent type, and proportion, pH, and voltage, achieving effective enantioseparation for fourteen model drugs without significantly prolonging migration times. The method was successfully applied to determine the enantiomeric purity of amlodipine besylate, with NMR and molecular modeling elucidating the chiral recognition mechanism [73]. This research highlights the potential of functionalized CIL as CSs in drug analysis.

Zhang and Ma developed a synergistic system for enantioseparation in CE using a CIL based on D-10-camphorsulfonic acid and CM-β-CD. The method demonstrated superior enantioselectivity for 16 chiral drugs, significantly improving resolution and the selectivity factor compared to a single CM-β-CD system. Optimization and statistical product and service solutions analysis confirmed the key role of CIL concentration in enhancing enantioseparation, with NMR experiments further validating the CIL's efficacy.

This system was successfully applied to determine chiral impurities in chlorpheniramine maleate samples [74].

A summary of studies focusing on the chiral purity analysis of pharmaceuticals utilizing CE methodologies, published over the last 15 years, is presented in Table 1.

Table 1. Studies of chiral purity control of pharmaceuticals using CE methodologies.

Year of Publication	CE Technique	Matrix	Analytes	CE Conditions	References
2010	MEEKC	bulk substance	ephedrine alkaloids	0.8% ethyl acetate, 1.0% SDS, 3.2% 1-butanol (*v*/*v*), 2.8% propan-2-ol (*v*/*v*), 92.2% 20 mM phosphate BGE, pH 3.0, 4.0% (*w*/*w*) S-β-CD, −15 kV, 20 °C	[68]
2010	NACE	bulk substance	4-amino-2,2-dimethyl-6-ethoxycarbonylamino-3,4-dihydro-2H-1-benzopyran	10 mM ammonium formate in methanol, 0.75 M formic acid BGE, 1.5 HDMS-β-CD, 5 mM EtChol NTf_2, 25 kV, UV detection 250 nm	[69]
2011	CZE	bulk substance	chloroquine (*S*-chloroquine chiral impurity in *R*-chloroquine)	100 mM phosphate BGE, pH 2.5, 30 mM SBE-β-CD, −25 kV, 20 °C, UV detection 225 nm	[30]
2011	CZE	bulk substance	RS86017–antiarrhythmic agent	25 mM phosphate BGE, pH 8.0, 20% acetonitrile (*v*/*v*), 28 mg/mL SBE-β-CD, 22 kV, 20 °C, UV detection 206 nm	[31]
2011	CZE	bulk substance, immediate release tablets, sustained release tablets	pramipexole (*R*-pramipexole chiral impurity in *S*-pramipexole)	50 mM phosphate BGE, pH 2.8, 25 mM CM-β-CD, 25 kV, 25 °C, UV detection 262 nm	[32]
2011	CZE	bulk substance	modafinil (*S*-modafinil chiral impurity in *R*-modafinil)	20 mM phosphate BGE, pH 7.5, 20% methanol (*v*/*v*), 20 mM SBE-β-CD, 20 kV, 25 °C, UV detection 225 nm	[33]
2012	CZE	bulk substance, tablet	citalopram (*R*-citalopram chiral impurity in *S*-citalopram)	25 mM phosphate BGE, pH 7.0, 1.6% (*w*/*v*) S-β-CD, −20 kV, 25 °C, UV detection 205 nm	[34]
2012	CZE	bulk substance, tablet	cetirizine (dextrocetirizine chiral impurity in levocetirizine)	50 mM borax BGE, pH 8.2, 1% (*w*/*v*) S-β-CD, 10 kV, 25 °C, UV detection 195 nm	[35]
2013	CZE	bulk substance, injection solution	ornidazole (dextrornidazole chiral impurity in levornidazole)	20 mM Tris-phosphate, pH 2.1, 2% (*w*/*v*) S-α-CD, 30 kV, 25 °C, UV detection 277 nm	[37]
2013	CZE	bulk substance, tablet	tolterodine, methoxytolterodine (*S*-tolterodine/methoxytolterodine chiral impurity in *R*-tolterodine/methoxytolterodine)	70 mM Tris-phosphate, pH 2.5, 3% (*w*/*v*) P-γ-CD, 20 kV, 25 °C, UV detection 200 nm	[38]

Table 1. *Cont.*

Year of Publication	CE Technique	Matrix	Analytes	CE Conditions	References
2014	CZE	bulk substance	amlodipine (*R*-amlodipine chiral impurity in *S*-amlodipine)	40 mM phosphate BGE, pH 3.5, 4 mM CM- β-CD, 30 kV, 25 °C, UV detection 237 nm	[39]
2014	CZE	bulk substance	glycopyrrolate (*S*,*R*-glycopyrrolate, *R*,*R*,-glycopyrrolate, *S*,*S*-glycopyrrolate chiral impurity in *R*,*S*-glycopyrrolate)	30 mM phosphate BGE, pH 7.0, 2% (*w*/*v*) S-β-CD, 20 kV, 25 °C, UV detection 210 nm	[40]
2014	CZE	tablet	valsartan (*R*-valsartan chiral impurity in *S*-valsartan)	25 mM phosphate BGE, pH 8.0, 10 mM A-β-CD, 30 kV, 30 °C, UV detection 205 nm	[41]
2014	CZE	bulk substance, pellets	omeprazole (*R*-omeprazole chiral impurity in *S*-omeprazole)	100 mM Tris-phosphate BGE, pH 2.5, 20 mM HP-β-CD, 1 mM sodium dithionite, 28 kV, 15 °C, UV detection 301 nm	[42]
2014	CZE CE-MS	capsule	duloxetine (*R*-duloxetine chiral impurity in *S*-duloxetine)	150 mM phosphate BGE, pH 3.0, 0.5% (*w*/*v*) HP-β-CD, 30 kV, 20 °C, UV detection 220 nm 150 mM ammonium formate BGE, pH 3.0, 0.5% (*w*/*v*) HP β-CD, 30 kV, 15 °C; sheath liquid, 3.3 µL/min of 80:20 (*v*/*v*) methanol/water with 0.1% (*v*/*v*) of formic acid	[70]
2015	CZE	injection solution	sulpiride (dextrosulpiride chiral impurity in levosulpiride)	5 mM Britton Robinson BGE, pH 3.45, 10 mM S-β-CD, 34 mM-β-CD, 14 kV, 16 °C, UV detection 214 nm	[43]
2016	CZE	tablet	vildagliptin (*R*-vildagliptin chiral impurity in *S*-vildagliptin)	75 mM Tris-acetic acid BGE, pH 4.75, 20 mM SBE-β-CD, 25 kV, 15 °C, UV detection 200 nm	[44]
2016	CZE	tablet	cinacalcet (S-cinacalcet chiral impurity in R-cinacalcet)	150 mM phosphate BGE, pH 2.5, 0,5% HP-γ-CD, 20% (*v*/*v*) methanol, 25 kV, 20 °C, UV detection 214 nm	[45]
2016	CZE	bulk substance	ambrisentan (*R*-ambrisentan chiral impurity in *S*-ambrisentan)	50 mM sodium acetate BGE, pH 4.0, 30 mM γ-CD, 25 kV, 20 °C, UV detection 200 nm	[46]
2016	MEKC	bulk substance, tablet	ambrisentan (R-ambrisentan chiral impurity in S-ambrisentan)	100 mM borate BGE, 100 mM SDS, pH 9.2, 50 mM γ-CD, 30 kV, 22 °C, UV detection 200 nm	[71]
2017	CZE	reference substance, injection solution	dextropromepromazine chiral impurity in levomepromazine	100 mM citric acid BGE, pH 2.85, 3.6 mg/mL HP-γ-CD, 25 kV, 15 °C, UV detection 253 nm	[47]

Table 1. *Cont.*

Year of Publication	CE Technique	Matrix	Analytes	CE Conditions	References
2018	CZE	bulk substance	lenalidomide (*R*-lenalidomide chiral impurity in *S*-lenalidomide)	30 mM phosphate BGE, pH 6.5, 30 mM SBE-β-CD, 12 kV, 10 °C, UV detection 210 nm	[48]
2017	CE	bulk substance	sitafloxacin (*R*,*S*,*R*-sitafloxacin, *R*,*R*,*S*-sitafloxacin, *S*,*S*,*R*-sitafloxacin chiral impurities in *S*,*R*,*S*-sitafloxacin)	15 mM phosphate BGE, pH 4.5, 20 mM γ-CD, 15 mM *D*-phenylalanine, 20 mM $CuSO_4$, 15 kV, 25 °C, UV detection 297 nm	[49]
2017	CZE	bulk substance	praziquantel (*S*-praziquantel chiral impurity in *R*-praziquantel)	50 mM phosphate BGE, pH 2.0, 15 mM S-β-CD, −15 kV, 25 °C, UV detection 210 nm	[50]
2018	CZE	capsule	pregabalin (R-pregabalin chiral impurity in S-pregabalin)	100 mM phosphate BGE, pH 2.5, 40 mg/mL HTM-β-CD, 15 kV, 25 °C, UV detection 220 nm derivatization with dansyl chloride	[51]
2018	CZE	tablet	cinacalcet (S-cinacalcet as chiral impurity in R-cinacalcet)	150 mM phosphate BGE, pH 2.7, 3.1 mM HP-γ-CD, 2% (*v*/*v*) methanol, 26 kV, 18 °C, UV detection 210 nm	[52]
2018	CZE	capsule	levomethorphan as chiral impurity in dextromethorphan	30 mM phosphate BGE, pH 6.5, 16 mg/mL S-β-CD, 14 mg/mL M-α-CD, 20 kV, 20 °C, UV detection 200 nm	[53]
2019	CZE	bulk substance	levomedetomidine as chiral impurity in dexmedetomidine	50 mM phosphate BGE, pH 6.5, 40 mg/mL S-β-CD, 10 kV, 17 °C, UV detection 200 nm	[54]
2019	CE	bulk substance	clopidogrel (R-clopidogrel chiral impurity in S-clopidogrel, acid hydrolysis product)	10 mM triethylamine–phosphoric acid, pH 2.3, 5% (*w*/*v*) S-β-CD, −12 kV, 20 °C, UV detection 195 nm	[55]
2019	CZE	bulk substance, tablet	dapoxetine (R-dapoxetine chiral impurity in S-dapoxetine, 3 chemically related impurities)	50 mM phosphate, pH 6.3, 45 mg/mL S-γ-CD, 40 mg/mL DM-β-CD, 9 kV, 15 °C, UV detection 230 nm	[56]
2019	CZE	capsule	lansoprazole (*S*-lansoprazole chiral impurity in *R*-lansoprazole) rabeprazole (*S*-rabeprazole chiral impurity in *R*-rabeprazole)	25 mM phosphate, pH 7.0, 10 mM SBE-β-CD, 20 mM γ-CD, 20 kV, 17 °C, UV detection 210 nm (lansoprazole) 25 mM phosphate, pH 7.0, 15 mM SBE-β-CD, 30 mM γ-CD, 20 kV, 18 °C, UV detection 210 nm (rabeprazole)	[58]

Table 1. *Cont.*

Year of Publication	CE Technique	Matrix	Analytes	CE Conditions	References
2019	CE	tablet	rasagiline (*S*-rasagiline chiral impurity in *R*-rasagiline)	50 mM glycine-chlorohydric acid, pH 2.0, 30 mM SBE-β-CD, −12 kV, 35 °C, UV detection 200 nm	[59]
2019	CZE	bulk substance	linagliptin (*S*-linagliptin chiral impurity in *R*-linagliptin)	70 mM sodium acetate, pH 6.1, 4.7 mM CM-β-CD, 28 kV, 25 °C, UV detection 200 nm	[60]
2020	CZE	reference substance, syrup	dextrodropropizine chiral impurity in levodropropizine, precursor	25 mM phosphate, pH 7.0, 23.5 mg/mL S-β-CD, 10% propan-2-ol (*v*/*v*), 16.5 kV, 16.3 °C, UV detection 200 nm	[61]
2022	CZE	bulk substance	tenofovir (S-tenofovir chiral impurity in R-tenofovir)	100 mM phosphate, pH 6.4, 45 mg/mL QA-β-CD, −18 kV, 22 °C, UV detection 257 nm	[63]
2022	CE	bulk substance	amlodipine (R-amlodipine chiral impurity in S-amlodipine)	20 mM phosphate, pH 2.5, 10 mM SBE-β-CD IL, 20% methanol (*v*/*v*), −12 kV, 25 °C, UV detection 237 nm	[73]
2023	CZE	bulk substance	apremilast (*R*-apremilast chiral impurity in *S*-apremilast)	50 mM phosphate, pH 7.0, 25 mM Succ-β-CD, −18 kV, 20 °C, UV detection 230 nm	[62]
2023	CZE	bulk substance, tablet	vildagliptin (*R*-vildagliptin chiral impurity in *S*-vildagliptin)	75 mM acetate, pH 4.5, 50 mM α-CD, 18 kV, 15 °C, UV detection 205 nm	[64]
2023	CZE	bulk substance, capsule	silodosin (*S*-silodosin chiral impurity in *R*-silodosin)	100 mM phosphate, pH 2.9, 40 mg/mL CM-β-CD, 28 kV, 17 °C, UV detection 200 nm	[66]
2024	CZE	bulk substance	tamsulosin (*S*-tamsulosin chiral impurity in *R*-silodosin), chemically related impurities	30 mM phosphate, pH 3.0, 40 mg/mL S-β-CD, 7 mg/mL CM-α-CD, −23 kV, 18 °C, UV detection 200 nm	[67]

5. Discussion

Over the past decade, the use of CE for analyzing chiral purity in pharmaceuticals has made significant advancements driven by technological progress and stricter regulatory requirements. Studies have demonstrated that CE techniques offer efficiency and flexibility comparable to traditional chromatographic methods like HPLC. This is largely attributed to the wide variety of CSs, including native and derivatized neutral and ionized CDs, which can be directly added to the BGE, facilitating effective enantioseparation through stereospecific interactions [14,15,19].

While CZE is commonly used for the chiral separation of pharmaceuticals, other techniques such as MEKC [71], MEEKC [68], and NACE [69] are also employed in certain circumstances. The method choice depends on the specific needs of the separation, such as the chemical nature of the analytes, their solubility, and the required resolution.

CDs, whether neutral or ionized, are the most frequently applied CSs in CE chiral separations. CDs are favored in chiral purity analysis due to their combination of versatility, efficiency, environmental friendliness, and cost-effectiveness, making them a powerful tool in pharmaceutical analysis. The use of a dual CD system, which combines both neutral and ionized CDs, is gaining popularity; this dual system enhances the separation efficiency and

selectivity by leveraging the complementary interactions of the two types of CDs with the analytes [25,43,53,56,58,67]. The use of CILs has seen a significant increase in recent years due to their unique properties that enhance chiral separation processes; these innovative solvents provide high thermal stability, tunable solubility, and the ability to form specific interactions with chiral molecules [21,69,73–75].

For optimizing methods in CE chiral separations, it is crucial to carefully select the type and concentration of CSs. This is in addition to optimizing standard factors in achiral CE such as BGE type and concentration, pH, the use of BGE additives, temperature, voltage, or injection parameters [14,19,76].

The development of chiral analytical procedures increasingly utilizes DoE, marking a shift from the traditional OFAT approach to a more systematic and efficient DoE methodology. The use of DoE in optimizing CE methods represents a major advancement; DoE allows for the systematic evaluation of multiple variables and their interactions, resulting in more robust and reproducible analytical methods. The results of developing methods utilizing DoE can provide insights into the relationship between analytical procedure variables and responses. This approach is especially crucial in the pharmaceutical industry, where the validation and consistency of methods are of paramount importance [28,47,49,51–56,58,60–62,64,66,67].

Nowadays, implementing QbD approaches is crucial for developing chiral CE procedures to evaluate the enantiomeric purity of pharmaceuticals marketed as pure enantiomers. QbD involves several critical steps: defining the ATP, such as accurately quantifying the main compound and its chiral impurity at the 0.1% level with high resolution in a short analysis time; identifying CPP, including factors like BGE concentration, pH, CS concentration, temperature, and voltage; and understanding how these parameters affect CMAs such as chiral resolution and analysis time. Finally, QbD establishes the design space, reflecting the experimental conditions under which the ATP is achieved [28,43,46,47,52,61,62,66,67,72,77].

Another notable trend is the development of CE methods that can simultaneously detect both enantiomeric and chemically related impurities in a single run. This approach not only enhances the efficiency of the analysis by reducing the number of required runs but also provides a more comprehensive assessment of the sample's purity. This holistic analysis capability is particularly valuable in pharmaceutical quality control, where understanding the full impurity profile is essential for ensuring the safety and efficacy of the final product [42,52,56,67].

Along with high flexibility, one of the biggest advantages of CE is the use of free CSs in BGE. This allows for example the migration of the analyte and CS in opposite directions increasing the separation selectivity, the possibility of using large amounts of CS and combinations of CS [14,18,78].

Another advantage of CE is its ability to modify the migration order of enantiomers through various mechanisms (using different CDs, adjusting pH, and modulating EOF). This feature becomes particularly important when detecting an enantiomeric impurity in the presence of much higher concentrations of the other enantiomer; in such scenarios, it is preferable for the enantiomeric impurity to migrate ahead of the major enantiomer [14,79].

CE, while efficient and versatile, faces several limitations, including challenges with reproducibility, column heating from high voltage, and the potential for adsorption of CSs to capillary walls. These issues can affect the consistency and quality of separations, requiring careful optimization and specialized strategies to mitigate their impact. Despite these challenges, CE remains a powerful tool in pharmaceutical analysis when these limitations are properly managed [14,18].

The stringent FDA and EMA regulations for chiral drugs require precise methods for assessing enantiomeric purity, and modern CE's increasing sensitivity and specificity, capable of detecting impurities sometimes lower than 0.1%, are essential for complying with these requirements [14,18,19].

CE methods are noted for their low environmental impact, aligning with "green chemistry" principles; the minimal use of organic solvents and small samples, reagents,

and CS requirements make CE a more sustainable option for pharmaceutical analysis, which is increasingly important in modern laboratory practices [14,16,20].

To enhance the capabilities of CE for chiral analysis, ongoing research into CSs and explaining and understanding their interaction mechanisms with enantiomers is essential; this research should focus on exploring functionalized and hybrid selectors, which can provide improved selectivity and stability. Combining CE with other analytical techniques, like MS and NMR, can provide comprehensive insights into the stereochemistry of pharmaceuticals, thereby enhancing the accuracy and depth of chiral analysis [80,81].

6. Conclusions

Since the 1990s, there has been a growing emphasis on quality criteria and chiral purity, driven by an understanding of chirality's impact on the pharmacological efficacy of optically active pharmaceuticals. Beginning in 1992, the FDA mandated the stereochemical evaluation of new optically active molecules, followed by similar requirements from the EMA in 1994. These regulations necessitate the thorough assessment of both racemic mixtures and individual enantiomers before their introduction into therapy. Consequently, developing analytical methods for enantiomer separation has become both essential and challenging in the pharmaceutical industry and its research.

While previous studies have established HPLC as the "gold standard" for chiral separation in pharmaceuticals, our review indicates that CE can offer comparable or even superior capabilities in certain contexts. The use of CDs as CSs in CE, for example, provides unique advantages in terms of selectivity and efficiency, which are sometimes limited in chromatographic techniques. Moreover, the rapid development and adaptability of CE methods make them well-suited for high-throughput analysis and real-time monitoring of enantiomeric purity. These findings have implications beyond pharmaceutical analysis, reflecting broader trends in analytical chemistry that highlight the importance of method flexibility, environmental sustainability, and regulatory compliance.

Our review indicates that CE is a robust and versatile tool for enantioselective separation, capable of meeting stringent regulatory requirements and facilitating future advancements in pharmaceutical analysis.

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Abbreviations

A-β-CD—acetyl-β-cyclodextrin; ATP—analytical target profile; BGE—background electrolyte, CD—cyclodextrin; CE—capillary electrophoresis; CEC—capillary electrochromatography; CIL—chiral ionic liquid; CE-β-CD—carboxyethyl-β-cyclodextrin; CM-α-CD—carboxymethyl-α-cyclodextrin; CM-β-CD—carboxymethyl-β-cyclodextrin; CMA—critical method attributes; CPP—critical process parameter; CQA—critical quality attribute; CS—chiral selector; CSP—chiral stationary phase; CZE—capillary zone electrophoresis; DM-β-CD—dimethyl-β-cyclodextrin; DoE—design of experiment; DPP-4—dipeptidyl-peptidase-4; DS—degree of substitution; EOF—electroosmotic flow; EMA—European Medicine Agency; Eur Ph—European Pharmacopoeia; FDA—Food and Drug Administration; GC—gas chromatography; HDAS- β-CD—heptakis(2,3-di-O-diacetyl-6-O-sulfo)-β-cyclodextrin; HDMS- β-CD—heptakis(2,3-di-O-methyl-6-O-sulfo)-β-cyclodextrin; HP-β-CD—hydroxypropyl-β-cyclodextrin;

HP-γ-CD—hydroxypropyl-γ- cyclodextrin; HPLC—high performance liquid chromatography; HTM-β-CD—heptakis(2,3,6-tri-O-methyl)-β- cyclodextrin; IS—internal standard; LOD—limit of detection, LOQ—limit of quantification; M-α-CD—methyl-α-cyclodextrin; M-β-CD—methyl-β- cyclodextrin; M-γ-CD—methyl-γ- cyclodextrin; MEEKC—microemulsion electrokinetic chromatography; MEKC—micellar electrokinetic chromatography; MODR—method operable design region; MS—mass spectrometry; NACE—non-aqueous capillary electrophoresis; NMR—nuclear magnetic resonance; NOESY—nuclear Overhauser effect spectroscopy; OFAT—one factor at time; P- γ-CD—phosphated-γ-cyclodextrin; PPI—proton pump inhibitor; QA-β-CD—quaternary ammonium-β-cyclodextrin; QbD—qQuality by Design; S-α-CD—sulfated-α-cyclodextrin; S-β-CD—sulfated-β-cyclodextrin; S-γ-CD—sulfated-γ-cyclodextrin; SBE-β-CD—sulfobutylether-β-cyclodextrin; SDS—sodium dodecyl sulphate; SFC—supercritical fluid chromatography; SNRI—serotonin and norepinephrine reuptake inhibitor; SSRI—selective serotonin reuptake inhibitor; Succ-β-CD—succinyl-β-cyclodextrin.

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