Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4), 532-538 | ISSN:2251-6727

Chiral Selector-Enhanced Chromatographic Separation of Chlorthalidone Enantiomers

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(Received: 04 August 2023 Revised: 12 September Accepted: 06 October)

KEYWORDS
beta cyclodextrin, chlorthalidone, enantioseparation, Chiral separation, Thin-layer chromatography

ABSTRACT:

Enantiomeric separation of (RS)-chlorthalidone has been achieved by thin-layer chromatography (TLC) using cyclodextrin, containing hydroxylic group as the chiral selector. For enantioseparation, cyclodextrin was used as a chiral additive in the stationary phase in a non-covalent mode and there was no chiral additive in the mobile phase; the native enantiomers were isolated and characterized. Cyclodextrin was also added to mobile phase and there was no chiral selector in the stationary phase. The spots were then isolated and characterized. The effect of the composition of the mobile phase, pH, and temperature was studied for the optimization of successful separation conditions. The spots were located in an iodine chamber.

INTRODUCTION

(*RS*)-Chlorthalidone (Fig. 1 Ctdn), a racemic mixture of 2-chloro-5- (1-hydroxy-3-oxo-1-isoindolinyl) benzenesulfonamide, is a diuretic medication that has been used extensively in hypertension treatments for more than 60 years, yet its polymorphism has only recently come to light. was accepted by the Food and Drug Administration for dealing with hypertension more than five decades ago, and it also acts in reducing blood pressure and reducing cardiovascular events. Ctdn's capacity to lower blood pressure is principally responsible for its potential to reduce cardiovascular events[1].

Many people have categorized Ctdn as a thiazide diuretic, probably due to its effects on the distal convoluted tubule of the kidney, where it prevents sodium reabsorption by inhibiting the Na+/-Cl symporter, a property it shares with true thiazides like hydrochlorothiazide (Hctz). Others have described the structural variations between Ctdn and real thiazides using the word thiazidelike[2]. However, Ctdn differs from thiazide diuretics in important ways, including its remarkable volume of distribution and effects on carbonic anhydrase.

When it comes to pharmacokinetics, Ctdn possesses qualities that make it a potent once-daily antihypertensive. The half-life with chronic dosing is estimated to be close to 50–60 hours[3]. Because Ctdn has a much broader volume of distribution than other thiazides—99% of the Ctdn dose is absorbed and bound by carbonic anhydrase within red blood cells[4], its prolonged half-life is likely the result of this. Theoretically, the high partitioning of Ctdn into erythrocytes generates a drug reservoir that permits drug discharge back into the plasma and keeps the drug level more stable. As is typical of shorter-acting (but dosed once-daily) diuretics[5], such an effect would promote a noticeably extended, low-level diuresis and so lessen the post-dose antinatriuretic phase that occurs when the drug level falls below the threshold for natriuresis. The fact that Ctdn is still efficacious when dosed less frequently than once day provides support for this functioning model[6,7]. Over the past 35 years, numerous research have investigated the effectiveness of Ctdn in decreasing blood pressure, both in dose-response studies and, more recently, in comparison studies versus the more widely prescribed diuretics. Although there is a distinct dose-response relationship, dosages between 12.5 and 25 mg per day are commonly employed because they maximize effectiveness while reducing electrolyte problems. Systolic blood pressure is reduced at these doses by roughly 8 to 18 mmHg[8-11]. Long-standing anecdotal opinions that Ctdn was more potent and effective than other diuretics in decreasing

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 532-538 | ISSN:2251-6727

blood pressure at the levels typically used in practice were confirmed in 2006 by a randomized, parallel-group, 8-week trial employing ambulatory blood pressure monitoring as the major objective[12].

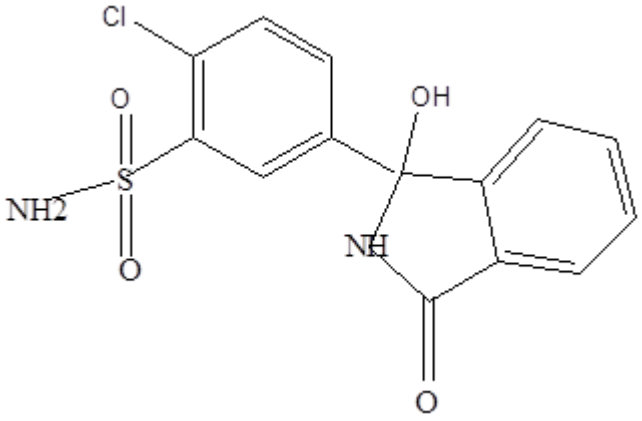

Figure 1. Structure of Chlorthalidone

Even while some of its solid-state characteristics, such powder X-ray diffraction characterisation, have been known from the beginning of the 1990s [13], the first solid-state structure wasn't revealed until the end of the previous decade.

2. Need for Enantioseparation:

Enantioseparation is the process of separating a racemic mixture into enantiomerically pure molecules. The organic structure is incredibly chirally selective, thus the enantiopure medicines are crucial. More than 500 medications are chiral, but only one of them—referred to as a eutomer—has any therapeutic efficacy, whereas the other, which is inactive or less potent, metabolizes in the body in a different way. As a result, chiral medicines must be divided into their potent and less potent isomers because both exhibit distinct pharmacokinetic and pharmacological effects[3,14].

In general, pharmacological and pharmacokinetic mechanisms lead to medication activity. There are many instances when pharmacological enantiomers differ in their bioavailability, distribution, metabolism, and excretion patterns, and where stereochemical factors are fundamentally important to the way they work and are disposed in biological systems. The U.S. Food and Drug Administration published a directive in 1992 stating that only the therapeutically active enantiomer of chiral drugs may be sold. Additionally, the pharmacological and metabolic routes of each enantiomer of chiral medicinal molecules should be individually investigated. In order to assess pharmacokinetics and the likelihood of interconversion as well as the absorption, distribution, metabolism, and elimination profile of individual enantiomers, manufacturers must develop quantitative assays for enantiomers individually in vivo samples. Manufacturers must track the pharmacological effects of each enantiomer separately in order to measure properties like dose linearity, effects of altered metabolic or excretory function, and drug-drug interactions if a candidate drug product is racemic with distinct pharmacokinetic profiles from its enantiomers[14,15]. Due to the nearly similar properties of enantiomers, chiral procedures are frequently needed for their separation, quantification, and even identification. Enantioseparation, which divides enantiomers based on their minute changes in characteristics, has a pivotal place among them. Enantioseparation can be utilized in simultaneous production of both enantiomers (dual-isomer recovery) or only the target enantiomer (single-isomer recovery).

Given that there is a market for both enantiomers, the former is frequently used in the production of chiral intermediates. Both enantiomers are ultimately retrieved using conventional methods after one is chosen by the chiral technology and the other is left behind. When just one enantiomer is commercially available, the latter method is typically used to produce end-use compounds or intermediates. The target enantiomer can also be chosen using chiral technology while the other enantiomer is purposefully racemized and recycled to produce the target enantiomer. The development of

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JCHR (2023) 13(4), 532-538 | ISSN:2251-6727

enantioseparation, which is regarded as one of the most important areas of research in both business and academia over the previous decades, has been encouraged by the large demand for enantiomerically pure products[16]. Enantioseparation has also attracted increasing attention in the fields of geology, geochronology, biochemistry, and materials science in addition to pharmaceutics and medicinal sciences.

Enantioseparation of Chlorthalidone-

Different β-cyclodextrins have been evaluated as chiral additives in the mobile phase for the chromatographic analysis of Ctdn enantiomers in a LiChrospher (12534 mm I.D.) column. 18 various parameters, including the type and concentration of b-cyclodextrines, the composition of the mobile phase (organic solvent percentage, kind of buffer, and pH), and flow rate were examined for their effects on enantioseparation. The most effective method for resolving Ctdn enantiomers was determined to be a 75:25 mixture of 0.1 M phosphate buffer and methanol, pH 4, containing 2% triethylamine (v/v) and 12.5 mM β-cyclodextrin, at a flow rate of 0.8 ml/min. Utilizing UV detection at 230 nm, linear calibration curves in the range of 0.5–20 mg/ml were achieved under these circumstances. For both isomers, the detection threshold was 50 ng/ml. By examining Ctdn in various pharmaceutical preparations, the usefulness of the reported assay has been evaluated. Additionally, authors have presented the biological sample application[14].

In order to perform enantioselective separations, supercritical fluid chromatography using chiral stationary phases is a common separation method. Supercritical fluid chromatography's key benefits include quick analysis times, little need for organic modifiers, lower prices, and greater environmental friendliness. It is reported that a unique approach for the separation of the two isomers of the widely prescribed diuretic medication Ctdn clearly exhibits the benefits of supercritical fluid chromatography. On the enantioselectivity and resolution of the enantiomers, the effects of temperature, back pressure, and the kind and quantity of organic modifiers were assessed. The optimized system, which consisted of a Chiralpak AD column, a mobile phase of CO2/ MeOH 50/50 (v/v), a temperature of 40C, a flow rate of 4.0 mL/min, and a back pressure of 120 bar, achieved the baseline separation in less than 2.5 minutes. In addition, the enantiomers of two medicines with commercial availability were found to contain Ctdn. The suggested technique is simple to adapt to a semi-preparative scale[15].

Enantiomeric separation of a medication combination made up of the chiral medicines atenolol and Ctdn. Using face-centered composite design, it was previously investigated how different variables affected the resolution of the enantiomers' peaks and the total run time, which is represented by the retention time of the last eluted peak. Twenty-two experiments were run with the chiral stationary phase type as a categorical component and the percentages of ethanol and diethylamine in the mobile phase as continuous factors. The chiral separation and measurement of the drug combination at 230 nm were carried out using a mobile phase of hexane, ethanol, DEA, and TFA (60:40:0.2:0.1%, v/v/v/v) injected at flow rate 1 ml/min onto Lux-Cellulose 2 stationary phase. The devised approach was effectively applied to the pharmaceutical formulation of this combination, and a reasonable recovery percentage was attained. In accordance with ICH (International Conference on Harmonization) norms, the procedure was also completely validated. This approach may be highly valuable and pertinent for use in quality control laboratories[16].

Using both univariate and multivariate calibration techniques, the concentrations of both Ctdn and spironolactone were determined simultaneously. The derivative ratio spectrum and zero-crossing approaches were used for univariate calibration. Poor results necessitated the use of multivariate calibration due to extensive spectrum overlap and the lack of wavelengths in derivative spectra that allowed one analyte to be differentiated and quantitated in the presence of the other. The ideal wavelength range and number of components for analysis were chosen using a combination of partial least-squares regression and an appropriate approach. A commercially available pharmaceutical preparation's simultaneous determination of Ctdn and spironolactone was done using the resulting method. By using high-performance liquid chromatography, (HPLC) the findings were verified[17].

The simultaneous assay of Telmisartan, Cilnidipine, and Ctdn in tablets has been developed and validated using

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JCHR (2023) 13(4), 532-538 | ISSN:2251-6727

a straightforward, accurate, and precise RP-HPLC method. On a BDS Hypersil C18, 250mm 4.6mm, 5m column, an isocratic RPHPLC method was developed. The mobile phase was Methanol: buffer (0.05M ammonium acetate), pH 5.0 with orthophosphoric acid (40:60), and the detection was done at 270nm using a waters 486 tunable absorbance detector. The drug was put through a forced deterioration experiment involving oxidative, oxidizing, acid, and base degradation. The linearity, accuracy, precision, and robustness of the approach were all confirmed. Telmisartan had a correlation coefficient of 0.9997, Cilnidipine had a correlation coefficient of 0.9989, and Ctdn had a correlation coefficient of 0.9989. The method was found to be linear in the concentration range of 20 to 60 g/mL. The test can be regarded as stability suggesting because the degradation products created as a result of stress experiments did not obstruct the detection of Telmisartan, Cilnidipine, and Ctdn[18].

The separation of enantiomers has been regarded as one of the most challenging tasks in chemistry from both an analytical and a preparative standpoint because of their similar chemical and physical properties. The interest in enantiomeric separation by HPLC has increased significantly over the past two decades thanks to the development and commercialization of numerous novel or better chiral stationary phases and chiral additives. The chiral selectors cyclodextrins and modified cyclodextrins are frequently employed. They can be utilized as chiral counter-ions, chiral mobile phase additives, or chiral stationary phases. The historical evolution of derivatized and underivatized cyclodextrins in HPLC and their numerous uses are discussed [19].

Material and Methods

Materials

Ctdn tablets (2.5 mg) were from Selco Enterprises Private Limited, Mumbai, India. Silica gel G with 13% calcium sulphate as binder, having lead, chloride and iron impurities up to 0.02%, with pH 7.0 in a 10% aqueous suspension was obtained from Merck (Mumbai, India). Other chemicals and reagents, of analytical grade, were obtained from Merck (Mumbai, India) and BDH (Mumbai, India). Some of the equipment used were, a direct Q (Millipore, France) water purifier dispensing system for supplying purified water, the terminal 740 (Indolab) pH meter, previously calibrated, for pH buffer adjustments, and SYSTONIC (Panchkula, Haryana, India) spectrophotometer (single beam, spectral bandwidth-2nm,10mm matched quartz cells) for recording UV-VIS spectra in methanol. Mobile phases and other solutions were submitted to ultrasonication with the help of an ultrasonic Elma Transsonic bath (model T700H, Tovatech, NJ, USA). Compact Quartz Polarimeter was from Friends (Ambala,Haryana,India) measuring range of optical rotation : +/- 180.

Extraction, isolation and purification of active API

Commercial 20 tablets of Ctdn were taken to extract about 50 mg of the API. All tablets were crushed/grinded to fine powder and was subjected to extraction in methanol by sonication for 15 min. The solutions were centrifuged at 3,000 rpm for 10 min; the residue was further extracted with methanol and centrifuged. The combined supernatant was concentrated in vacuum and left to cool until crystals appeared. The mother liquor was decanted and the crystals were dried. The samples were further purified by recrystallization with methanol and were used as standard analytes[20].

Preparation of Standard Solutions

Stock standard solutions with concentrations of 1 mg mL^{-1} were prepared using methanol as the solvent. Working standard solutions were freshly prepared by dilution with methanol to obtain solutions having concentrations of 0.1 mg mL^{-1}.

Procedures

Development of chromatograms

The analysis was performed on pre-coated 20 cm × 20 cm silica gel 60 F254 aluminium sheets Solution of Ctdn was spotted (10µL) using Hamilton syringe, on thin-layer plates 2 cm above the margin. Chromatograms were developed using different solvents such as, acetonitrile, methanol, ethyl acetate, toluene, and glacial acetic acid, in various systematic compositions (binary, ternary and quaternary) to achieve the enantioseparation. Chromatograms were developed in cleaned, dried and paper-lined rectangular glass chambers pre-equilibrated with the mobile phase at different temperatures (17±2, 25±2, and 32±2 °C) for 15 min under the controlled conditions. The

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JCHR (2023) 13(4), 532-538 | ISSN:2251-6727

chromatograms so developed were dried in air for 10 to 15 min. The spots were located as dark brown spots in an iodine chamber[19,21].

Isolation of API from TLC plates

The Ctdn was also spotted on Homemade TLC plates (10 × 10 cm with 1.5 mm thickness) prepared by spreading slurry of silica gel G (25 g) in distilled water (50 mL), with a Stahl-type applicator and drying the plates at room temperature and then activating them for 8-10 h at 60 ±2 °C. In one of the methods, the slurry was prepared by dissolving requisite amount of b- CD in the slurry. Spots corresponding to each enantiomer (separated from the racemic mixture) were marked on the chromatograms and iodine was allowed to evaporate. Silica gel of each of the spots was scraped (from nearly 10 chromatograms); the combined silica gel for each spot was extracted by sonication with methanol (5 mL, five times). The combined extracts for each of the analytes were filtered through Whatman filter paper grade number-1 and the residues were further treated thrice with the same solvent mixture and filtered. The combined extracts (for each case) were then dried and characterized[20,22].

Method Optimization

The effect of the concentration of impregnating reagents with silica-gel and in the mobile phase was investigated. It was observed that the best resolution was at 0.3% of the impregnating reagent. As the concentration was decreased to 0.1% and 0.2%, the resolution became poorer in all the solvent combinations. An increase in the concentration of the impregnating reagent to 0.5% also resulted in poor resolution[23,24].

The chromatographic conditions were also optimized by spotting the drug on TLC plates and developing different solvent systems in order to achieve the best separation. Initially, a combination of Toluene-ethyl acetate-methanol- glacial acetic acid- b- CD in different ratios was tried. After trying several combination ratio, best separation was observed with Toluene-ethyl acetate-methanol-glacial acetic acid – 5mM CD (5:3:2:0.1:1:0). The average R_F values of enantiomers of Ctdn using both the methods is tabulated in Table 1.

Chromatographic plates showing the separation of enantiomers of Ctdn is shown in fig 2

a) b)

Fig. 2 Photographs of TLC plates, showing separation of enantiomers. a) TLC plates prepared by added chiral selector to the slurry. b) the separation of enantiomers where chiral selector was added to the MP. Chromatographic conditions are mentioned in Table 1.

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Method	Mobile phase	Rf (lower spot)	Rf (upper spot)	Resolution (Rs)
Adding chiral selector to the slurry	Toluene-ethyl acetate-methanol-glacial acetic acid (5:3:2:0.1)	20	34	2.6
Adding chiral selector to the mobile phase	Toluene-ethyl acetate-methanol-glacial acetic acid: CD (5:3:2:0.1: 1.0)	22	37	2.8

Results and Discussion

CD and their derivatives have been extensively used as chiral selectors for HPLC chiral separation due to their natural chirality and ability to form inclusion complex with molecules via hydrophobic cavity[25]. It was reported that the combination of hydrophobic interactions and steric effects from the substituents present on the cavity entrance are believed to be responsible for the observed enantioselectivity in reversed-phases HPLC. The chiral recognition of CD CSPs under reverse-phase conditions is thought to be driven by the inclusion complexation between the hydrophobic moiety of analyte and the relatively non-polar interior of the CD cavity. Therefore, the dimension of CD-cavity is likely to have substantial effects on the enantioseparation ability of CD-bonded CSPs under reversed-phase conditions. Under normal-phase conditions, however, the CD-cavity is more likely to be occupied by the non-polar molecules of the mobile phase and the chiral recognition ismainly attributed to the interaction and hydrogen bonding between sites provided by the aromatic and carbonyl substituents on the derivatized CD. Similar recognition mechanism may be applied to explain the enantioseparation of Ctdn on β-CD. Their bicyclic moiety containing the chiral center was apt to be “tight-included” into the cavity of β -CD, which resulted in their separation on β-CD[26,27].

Conclusion:

The method reported herein provides a simple, rapid, and effective approach in the planar mode for separation of enantiomers of Ctdn, which can be realized even in a small laboratory. The method may be applied for successful resolution of a variety of pharmaceuticals and other organic racemic mixture.

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