Est. 1984

ORIENTAL JOURNAL OF CHEMISTRY
An International Open Access, Peer Reviewed Research Journal

www.orientjchem.org

ISSN: 0970-020 X
CODEN: OJCHEG
2023, Vol. 39, No.(6):
Pg. 1714-1719

Direct Enantiomeric Separation of Indapamide by Thin Layer Chromatography using β-cyclodextrin as Chiral Selector

MANJU BALA[1], SONIKA SETHI[1]* and PRIYANKA SHARMA[1]

[1]School of Engineering and Sciences, GD Goenka University, Gurgaon, Haryana, India.
*Corresponding author E-mail: sonikabatra211@gmail.com

http://dx.doi.org/10.13005/ojc/390636

(Received: September 04, 2023; Accepted: November 02, 2023)

ABSTRACT

A novel economic thin layer chromatographic method for stereoselective separation of racemic mixture of (RS)-Indapamide and determination of their enantiomers was done. The method was based on using normal TLC plates and cyclodextrin with hydroxylic part, was used as the chiral selector. Cyclodextrin was used as an additive in silica gel to prepare a TLC plate which was a non-covalent bonding, and there was no chiral substance added in the solvent system. The mobile phase was toluene-ethyl acetate-MeOH-glacial AcOH (6:4:1:0.1). Cyclodextrin was also added to mobile phase, and there was no chiral selector in the stationary phase. The mobile phase was a mixture of toluene-ethyl acetate-MeOH-glacial AcOH–5mM CD (6:4:1:0.1:1.0). The spots were then isolated and identified. The impact of the components of mobile phase, temperature, and pH was studied for the finding the best separation conditions. The spots were sited in a chamber which had Iodine granules.

Keywords: Thin-layer chromatography, β-cyclodextrin, Indapamide, Diuretics, Chiral separation.

INTRODUCTION

Indapamide (Indpd, Fig. 1) is antihypertensive agent and a mild diuretic. Chemically, sulphonamide diuretic drug, Indpd is '1-(4-chloro-3-sulfamoylbenzamido)-2-methylindoline'. Indpd has a moiety soluble in lipid, which differentiate the activity of Indpd from other diuretics. Indpd has been exposed to be an effective long acting antihypertensive agent when used alone or along with other healing agents. Indpd has been effectively combined with beta-blockers, methyldopa and other antihypertensive reagents.[1]

Indpd is known to work for high blood pressure, and for the reduction in edema as well as a reduction in total peripheralopposition. It is generally used in the treatment of hypertension, as well as decompensated cardiac failure.[2]

Indpd is soluble in MeOH, AcOH and EtOAc; it is very slightly soluble in $CHCl_3$.

Need for Enantioseparation

Drug activity is primarily influenced by pharmacological and pharmacokinetic factors. In numerous cases, drug enantiomers demonstrate variations in bioavailability, distribution, metabolism, and excretion, with stereochemical properties playing

a crucial role in their interactions within biological systems.[3,4] In nineteenth century, the U.S.F.D.A shared guidelines stipulating that only therapeutically active enantiomer of a chiral drug can be approved for market distribution.[5] Consequently, manufacturers are required to comprehensively investigate each enantiomer of chiral drug compounds, studying their respective pharmacological and metabolic pathways independently.[6] To facilitate this assessment during drug development, manufacturers must establish quantifiable assays for distinct enantiomers in in vivo samples, enabling the evaluation of their pharmacokinetics. This evaluation helps assess the probability for interconversion and the way it is absorbed, distributed, metabolised, and eliminated in the body. In cases where a candidate drug product is racemic and exhibits distinct pharmacokinetic profiles among its enantiomers, producers must monitor the pharmacological effects of each enantiomer separately.[7] Given that enantiomers typically possess nearly identical properties, specialized chiral methods are often necessary for their separation, quantification, and sometimes identification. Among these methods, enantioseparation, which leverages subtle differences in properties to separate enantiomers, plays a pivotal role. Enantioseparation can be advantageous for simultaneous production of both enantiomers (dual-isomer recovery) or when producing only the desired target enantiomer (single-isomer recovery).[8] the former approach is commonly employed in the production of chiral intermediates, where both enantiomers find their way to the market. In this process, chiral technology selects one enantiomer while leaving the other behind, and both enantiomers are subsequently recovered through conventional means. Conversely, the latter approach is typically utilized for the manufacture of end-use chemicals or intermediates when only one enantiomer is marketable. In such cases, chiral technology can selectively isolate the target enantiomer while intentionally racemizing the other enantiomer and recycling it during the selection process to yield the desired target enantiomer.[9] The increasing demand for enantiomerically pure products has driven significant growth in enantioseparation, making it a key area of research in both industry and academia over the past few decades. In addition to pharmaceuticals and medicinal sciences, enantioseparation has gained recognition in fields such as geochemistry, geochronology, biochemistry, and materials science.[10] To obtain enantiomerically pure compounds by separation of racemic mixture is known as enantioseparation.The enantiopure drugs are crucial because the organic structure is amazingly chirally selective. More than five hundred medicines are chiral however the medicine activity exist in just one compound, termed as eutomer, whereas the opposite compound that is inactive or less potent metabolizes by adifferent means within the body.Therefore, it is required to separate chiral drugs into their effective and less effective isomers as they both show different pharmacokinetic and the pharmacological effects.[6]

Enantioseparation of Indpd

Literature review reveals that different analytical approaches like UV spectroscopy, RP-HPLC, and HPTLC have been described for the enantioseparation of Indpd, individually and in combination with other drugs.[11] HPLC enantioseparation of Indpd was completed using an ovomucoid protein based Chiral Selector (CS) as stationary phase performed on HPLC instrument from Agilent. An Ultron ES Ovomucoid, column was used for the separation of the two optical isomers.[12] Simultaneous enantioseparation of Indpd and perindopril tert-butylamine (PER) enantiomers was established successfully by HPLC using ovomucoid as CS. The separation of enantiomers was performed on an Agilent 1100 Series HPLC system, with UV-Visible detector using an Ultron ES Ovomucoid column. The effects of buffer pHs, amount of organic modifier, the injection volume and the column temperature have been studied for resolution and selectivity. Good results were found from this analysis signifying that the proposed method was appropriate for the study of both the drugs in therapeutic fixed-dose combinations.[13] In another study, stereoselective method for the estimation of Indpd enantiomers in whole blood has been defined using HPLC. Resolution of enantiomers was attained on a "cellulose tris (3,5-dichlorophenylcarbamate)" column known as Chiralpak IC, and the mobile phase (MP) consisting of n-C_6H_{12} and CH_3-CH(OH)CH_3 (70:30, v/v).[14] Test methods have been reported for determination of various diuretics (say, Indpd) in whole blood, plasma, urine or serum after liquid-liquid extraction followed by HPLC with UVdetection.[15] These methods have been monotonous for whole blood samples with UV detection, and required a control blank and one

or the other internal standard. Chromatographic procedures and methods for bioequivalence study, drug screening and enantioseparation of Indpd have been reviwed.[16]

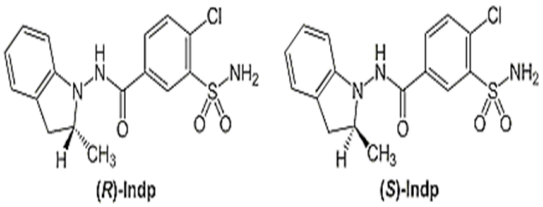

Fig. 1. Structure of Indpd(adapted from Ref (16))

Liquid chromatography (LC) offers both direct and indirect methods for enantioseparation, each with its own advantages and limitations depending on factors such as sample quantity and laboratory resources. Numerous assessments and articles have explored issues related to enantioseparation modes, namely 'direct' and 'indirect,' for a wide range of racemic compounds. Among the various LC methods available, TLC stands out for its simplicity and cost-effectiveness. It provides the added benefit of producing chromatogram images as tangible evidence of successful separation. LC methods for enantioseparation encompass both direct and indirect approaches, involving practical applications and the evaluation of multiple chiral stationary phases (CSPs), chiral derivatization reagents (CDRs), as well as utilization of chiral mobile phase additives (CMPA), impregnation, and ligand exchange techniques. These methods have been extensively studied for the separation of Indpd enantiomers.[17] Hence, the literature references which have been cited therein, are not being repeated here.

Structure of Cyclodextrin

Cyclodextrin (Fig. 2, CDs) is formed during bacterial digestion of cellulose. The structure of CD has been explained in the previously published reports and hence is not being discussed here.[18] CDs have been used as CSs in capillary electrophoresis (CE) as well as HPLC and is characterized as the most commonly used type of CS for a broad application range because of its several advantages like its structure low cost, high solubility and low UV absorbance. The separations using CDs as CSs can be done in the polar organic mode, the reversed-phase mode, and the Normal phase (NP) mode.[19-21]

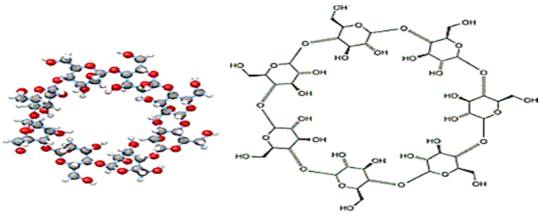

Fig. 2. The structure of β-CD (adapted from Ref (21))

MATERIAL AND METHODS

Materials

Lozol tablets of Indp (2.5 mg) were from Selco Enterprises Private Limited, Mumbai, India. Silica gel G with 13% $CaSO_4$, with Pb, chloride and Fe impurities up to 0.02%, with pH 7.0 was acquired from Merck (Mumbai, India). Other reagents, of analytical grade, were acquired from BDH and Merck (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 MeOH. MPs and other solutions were submitted to ultrasonication with the help of an ultrasonic Elma Transsonic bath (model T700H, Tovatech, NJ, USA). Compact Quartz Polarimeter from Friends (Ambala, Haryana, India) measuring range of optical rotation: +/- 180, Division Value: 1 degree, Least count: 0.05 degree or less, Monochromatic light source: 580-590nm, Stabilization time (approx.): 5 minutes.

Extraction, isolation and purification of active API

Commercial 20 tablets of Indpd were taken to extract about 50 mg of the API. All tablets were crushed/grinded to fine powder and were subjected to extraction in MeOH by sonication for fifteen minutes. The solutions were centrifuged at 3,000 rotations per minute for ten min; followed by extraction with MeOH and again 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 MeOH and were used as standard analytes. The purity was checked by TLC (a single spot was obtained) and by the melting point.

Preparation of Standard Solutions

Stock standard solutions were prepared

using MeOH with concentrations of 1 mg mL^{-1}. Solutions for the experiment were freshly prepared by diluting it with MeOH to obtain solutions having concentrations of 0.1 mg mL^{-1}.

Procedures

Development of chromatograms

The analysis was performed on pre-coated standard 20 cm×20 cm silica gel 60 F254 aluminium sheets. Working solution of Indpd was spotted (10 µL) using Hamilton syringe, on plates approx. 2 cm above the margin. Chromatograms were developed using different solvents such as, acetonitrile, MeOH, ethyl acetate, toluene, and glacial AcOH, in different compositions of two, three and four solvents, to achieve the enantioseparation. Chromatograms were developed in chambers made of glass pre-equilibrated with the m.p. at varying temperatures (18±2; 25±2, and 30±2°C) for 15-20 minute. The chambers were clean, dry and paper-lined. The plates so developed were put to dry in air for 12 to 18 minute. The spots were located in a chamber which had iodine granules.

Separation of API from TLC plates

The Indpd was also spotted on homemade TLC plates (10×10 cm with a thickness of 1.5mm) prepared by thinning out slurry made up of silica gel G (~25 g) in distilled H_2O (50 mL), with a Stahl-type applicator and drying the plates at RT 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 β-CD in the slurry and in the other, CS was added to the MP and slurry was prepared only in MeOH. Spots corresponding to each enantiomer (separated from the racemic mixture) were marked on the plates and iodine on the spots was allowed to evaporate. Silica gel of each of the spots was scratched (from 12 plates); the combined silica gel scrapped from the plates was extracted by sonication with MeOH (5 mL, five times). The combined extracts for each of the enantiomers were filtered and the residues were further treated with MeOH and filtered. The combined extracts for both the enantiomers were then dried and characterized.

Method Optimization

The impact of the concentration of CD with silica-gel and in the MP was investigated. The best resolution was obtained at 0.5% of the β-CD. The resolution became poorer as the concentration was decreased to 0.4% and 0.3%. Also, increasing the conc to 0.6% resulted in reduced resolution.

The experimental conditions were optimized by spotting the drug on TLC plates and using different m.p. so as to achieve the best separation. Initially, a combinationof $PhCH_3$-ethyl acetate-MeOH-glacial AcOH-β-CD in different ratios was tried. After trying several combination ratios, best separation was observed with Toluene-ethyl acetate-MeOH-glacial AcOH–5mM CD (6:4:1:0.1:1:0). The average RF values of enantiomers of Indpd using both the methods is tabulated in Table 1.

TLC plates showing the separation of enantiomers of Indpd using both the methods is shown in Fig. 3. The separation of enantiomers uisng the TLC plates prepared by added CS to the slurry is shown in Fig. 3a whereas, Fig. 3b, represents the separation of enantiomers where CS was added to the m.p. The results have been compliled in Table 1. Resolution was slightly better in the later case.

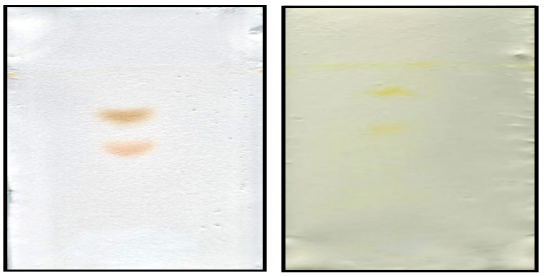

Fig. 3. Photographs of TLC plates, showing separation of enantiomers. a) TLC plates prepared by added CS to the slurry. b) the separation of enantiomers where CS was added to the MP. Chromatographic conditions are mentioned in Table 1

Table 1: Data of separation of Indpd enantiomers on TLC plate

Method	m.p.	R_F(lower spot)	R_F(upper spot)	Resolution (Rs)
Adding CS to the slurry	$PhCH_3$-EtOAc-MeOH-gl AcOH (6:4:1:0.1)	23	45	2.76
Adding CS to the MP	$PhCH_3$-EtOAc-MeOH-glacial AcOH: CD (6:4:1:0.1: 1.0)	34	52	2.85

RESULTS AND DISCUSSION

CD and their derivatives, being chiral in nature and are able to form an inclusion complex with analyte molecules using their cavity which is hydrophobic in nature, therefore are used as CSs for HPLC.

The observed enantioselectivity is due to the combined effect of steric effects and the hydrophobic interactions. Size of CD-cavity is having impact on the enantioseparation ability of CD-bonded CSPs under RP conditions.[22]

Characterization of the separated enantiomers

Chiral compounds, also known as optical isomers, differ in the way they rotate a plane of polarized light.The measurement specific rotations of optical isomers, can be a powerful analytical tool for analyses of chiral compounds. The optical rotation is a characteristic property of compound, and it is related to its concentration in the solution.[33,34]

The determination of specific optical rotation is the characteristic requirementof drugs administered as the pure enantiomer, according to the pharmacopoeias. The extracted enantiomers were subjected to polarimetry. The values of specific rotation were found to be -0.0190 for one enantiomer and +0.0190 for the other enantiomer dissolved in ethanol. These were in line with the reported values. Thus validating that the enantiomers were separated with the lower spot being the (+)–enantiomer and the upper spot corresponds to the (-)–enantiomer of Indpd.

CONCLUSION

With the growing demand, for enantiomerically pure products, nowadays, many industrial productions follow a less expensive conventional chemical synthesis of the racemic/enantiomeric mixture followed by enantioseparation. Enantioseparation using HPLC is expensive as it requires expensive instrument with investment in columns. However TLC is an easy and very cost effective technique, which can be utilized for method development.[23-24]

The enantioseparation method described in this article provides a quick, convenient and effective approach in the planar mode for separation of enantiomers of Indpd, which can be practiced even in a small scale laboratory. The method described, may further be applied for successful separation of enantiomers of a variety of racemic pharmaceuticals of similar structure with minimal optimization.

ACKNOWLEDGEMENT

The authors are grateful to GD Goenka University, Gurugram for the laboratory support to carry out the experimental work.

Conflict of interest

There is no conflict of interest.

REFERENCES

1. Chaffman, M.; Heel, R.C.; Brogden, R.N.; Speight, T.M.; Avery, G. S. *Drugs.*, **1984**, *28*(3), 189-235.
2. Mroczek, W.*J. Pharmacotherapy.*, **1983**, *3*(2 Pt 1) 61-7.
3. McConathy, J.; Owens, M.J. *J. Clin. Psychiatry.*, **2003**, *5*(2), 70-73.
4. Burke, D.; Henderson, D.J. Br. *J. Anesth.*, **2002**, *88*(4), 563–576.
5. GUIDANCE DOCUMENT,Development of New Stereoisomeric Drugs,Center for Drug Evaluation and Research., **1992**.
6. Brooks, W.H.; Guida, W.C.; Daniel, K.G. *Curr Top Med Chem.*, **2011**, *11*(7), 760-70.
7. Peter, L.; Robert, H.; Phil, R.; Pierre-Louis, T. *J. Vet. Pharmacol. Ther.*, **2012**, *35*, 17-30.
8. Nguyen, L. A.; He, H.; Pham-Huy, C. *Int J Biomed Sci.*, **2006**, *2*(2),85-100.
9. Aboun-Enein, H.Y.; Ali, I. Aboun-Enein HY, Ali I, editors. New York: Marcel Dekker., **2003**, 1–20.
10. De, K.N.; Clark, C.P.; Kohler, I. *Electrophoresis.*, **2021**, *42*, 38-57.
11. Sethi, S.; Martens, J.; Bushan, R. Acta Chromatographica. https://doi.org/10.1556/1326.2023.01123).
12. Carje, A.G., Ion, V., Muntean, D.L., Hancu, G., Balint, A., Imre, S. *Farmacia.*, **2016**, *64*, 181–186.

13. Tao, Y.; Wang, S.; Wang, L.; Song, M.; Hanga, T. *J. Pharm. Anal*., **2018**, *8*, 333–340.
14. Du, B.; Pang, L.; Li, H.; Ma, S.; Li, Y.; Jia, X.; Zhang, Z. *J. Chromatogr*., B. **2013**, *932*, 88–91.
15. Choi, R. L.; Rosenberg, M. E.; Grebow, P.; Huntley, T. E. *J. Chromatogr*., **1982**, *230*, 181–187.
16. Brent, M. R.; Dadgar, D.; Lalande, M. *J. Chromatogr*., **1993**, *614*, 293–298.
17. Tero-Vescan, A.;Hancu, G.;Oroian, M.;Cârje, A. *Adv Pharm Bull*., **2014**, *4*(3), 267-72.
18. Loftsson.; Thorsteinn Jarho.; Pekka & Másson, Már & Järvinen, Tomi., *Expert Opin Drug Deliv*., **2005**, *2*, 335-51.
19. Szejtli, *J. Chem. Rev*., **1998**, *98*, 1743–1753.
20. Okamoto, Y.; Kawashima, M.; Hatada, K. *J. Am. Chem. SOC*., **1984**, *106*, 5357-5359.
21. Moiseev, R.; Morrison, P.; Steele, F.; Khutoryanskiy, V. *Pharmaceutics*., **2019,** *11*, 321.
22. Mehta, A.C.; *J Clin Pharm Ther*., **1990**, *15*, 313-323.
23. Sherma, J. Thin-Layer Chromatography. In Encyclopedia of Analytical Chemistry (eds R.A. Meyers and J.G. Dorsey)., **2006**.
24. Gupta, M.K.; Ghuge, A.; Parab, M.; Al-Refaei, Y.; Khandare, A.; Dand, N.; Waghmare, N.; *Curr. Issues Pharm. Med. Sci*., **2022**, *35*, 224-228.