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Enantiomeric Separation of Selenomethionine Using Liquid Chromatographic Techniques and its Biological Importance: A Review

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Abstract

Selenomethionine (2-amino-4-methylselanylbutanoic acid) is a Se-containing naturally occurring chiral amino acid and is well known for its dietary and biological importance and has been used as a source of Se in humans, other mammals and plants. L-SeMet is better absorbed and better incorporated into the body than any other known form of selenium. This is due to the presence of active functional groups carboxyl (-COOH) and amino groups ($-NH_2$) in their unique and simple structure. In this review attempts are being done the enantiomeric separation of Selenomethionine by using chromatographic techniques and deals with the application of: thin layer chromatography by direct method using chiral selector as, chiral inducing reagents and chiral impregnating reagent. High-performance liquid chromatography, by direct method using chiral stationary phase columns and, by indirect method using chiral derivatizing reagents.

Keywords: Selenomethionine; Enantiomeric separation; Chiral selector; Chiral derivatizing reagent; High-performance liquid chromatography; Thin layer chromatography

Abbreviations: CSP: Chiral Stationary Phase; TLC: Thin-Layer Chromatography; PDA: Photodiode Array Detector; CMPA: Chiral Mobile Phase Additives; CIR: Chiral Inducing Reagent.

Introduction

Selenomethionine (2-amino-4-methylselanylbutanoic acid, SeMet) (Figure 1) is a Se-containing naturally occurring chiral amino acid. L-SeMet is a natural food form of Se for human while its enentiomer (D-SeMet) is toxic at high level and its bioavailability as inorganic Se by metabolism is only up to one-fifth of L-SeMet [1]. Its importance as Se source in humans, others mammals and plants species has been discussed in books and reviews [2-4]. There may be negative impact of Se deficiency, on the immune system [5] and leads to higher risk in case of infection with HIV [6]. Some physiological functions, related to central nervous system [7], cardiovascular system [8], reproduction ability [9] and inflammatory processes [10] are influenced by selenium.

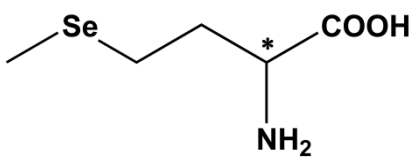

Figure 1: (2-amino-4-methylselanylbutanoic acid, SeMet).

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Since our body is constituted by chiral compounds and stereo selective for drugs racemic mixture, therefore whatever drug patient taking for curing of disease, if racemic then these two enentiomer present in drugs may have different activity or these can be considered as different compounds. Many biologists and clinical pharmacologists dealing the drugs with enantiomeric mixtures of isomers and a physician, unaware of different activity of enantiomers, presenting such a drug in racemic mixture under a brand name may make mistakes [11]. Therefore, pharmacologists are informed by regulatory agencies and ask to present full pharmacodynamics and pharmacological information of both the enantiomers. Because in certain cases, there may be side effects or even toxic effects due to the presence of less reactive or unwanted enantiomer. Therefore, efficient methods are always required to resolve racemic mixtures and, to control and to determine enantiomeric purity of the drugs.

There is immense importance of enantiomeric separation in various fields from academic and industrial point of view. Two basic strategies ‘‘direct approach’’ and ‘‘indirect approach’’ have evolved in recent years for enantiomeric separation. The direct approach do not require derivatization; in thin layer chromatography separation can be achieved by using chiral selector as chiral mobile phase additives, chiral impregnating reagent and chiral inducing reagent, and in high-performance liquid chromatography by using chiral selector as chiral stationary phase. Chiral selector produces chiral environment in stationary phase *in situ* formation of transient diastereomers between an analyte and the enantiomer of pure chiral selector. In indirect method, enantiomers of analyte species converted in to diastereomers by reacting them with chiral derivatizing reagents and then daistereomers can be separated in achiral environment by using chromatographic techniques. Certain basic approaches and principles of planar and column chromatographic techniques for enantiomeric separation of SeMet are already reported but no any methods is described for the separation of enantiomers of DL-SeMet by chromatographic method using direct and indirect approaches. The present review article is focused on the use of liquid chromatographic techniques for the enantiomeric separation of DL-SeMet.

Direct approach

The efficiency of enantiomeric separation in terms of “resolution” using direct approach depends on the stereo selective environment either produced by incorporating a suitable chiral selector at an appropriate stage or by using chiral stationary phase (CSP). In thin-layer chromatography (TLC) chiral environment can be produced by using (a) TLC plate or column with the chiral selector, (b) adding the chiral selector to the mobile phase, or (c) impregnating the chiral selector on stationary phase of TLC. The material mentioned at (a) is chiral owing to its own structure (e.g. cellulose, α_1-acidglycoprotein and amylase tris (3,5-dimethylphenylcarbamate) etc.) while the material mentioned at (b) chiral environment is created by mixing the chiral selector and with mobile phase and flow of this mixture prior to separation. In situation (c) chiral environment is prepared by bonding the chiral selector of interest to reactive groups of inert support.

In all the cases, chiral selector interacts with enantiomers of analyte of interest and forms the transient diastereomers on the surface of inert support. The basic principle of separation of these enantiomers is based on their hydrophobic/hydrophilic interactions with the inert support/mobile phase. The enantiomer forming most stable diastereomers will be most retained with stationary phase and may be eluted later while the another enantiomer forming less stable diastereomer most interact with mobile phase and elute first. Direct methods have been applied by Martens & Bhushan [12]. For the separation of enentiomers which are important in various fields like pharmaceutuical and biomedical analysis and, synthetic and mechanistic study.

Enantiomeric Separation by TLC

Following methods are used

Impregnation: In impregnated TLC technique a suitable chiral selector (CS) is mixed with the adsorbent without covalently affecting its inert character, owing to application of samples of enentiomers of interest on the plate for their separation. There are certain methods have been used for impregnation of TLC: mixing the chiral selector with the inert support during the formation of slurry of these two components; immersing the plain plate into an appropriate solution of the impregnating reagent as ascending or descending development; spraying the solution of the impregnating reagent on the plate; and exposing the thin layer to the vapors of the impregnating reagent. The methods used for impregnation also play an important role in the resolution of the enantiomers, as in immersion method; peeling of the thin layer of inert support, and in spraying method; less uniform dispersion of the chiral selector than the method of mixing of chiral selector with inert support or immersion [12] may affect the results up to measurable level.

This is an inexpensive method and provides wide choice of chromatographic conditions for enantiomeric separation from racemic mixture of variety of compounds. Enantiomeric separation of DL-SeMet has been carried out using (–)-quinine [13], as chiral impregnating reagent by two approaches (CS mixed in the slurry of silica gel before making the plates and ascending development of the plate in the solution of CS) and chromatographic data in terms of enantiomeric resolution and limit of detection (LOD) are mentioned in Table 1.

S. No.	CSP[a]/CDR[b]/Chiral Selector[c] /CIR[d]	Technique used	Resolution time (t_R/k/run time)	LOD	R_S	Reference
1	*β*-cyclodextrin[a]	i	40.26/42.74 min (t_{R1}/t_{R2}) 17.23/18.91 min (t_{R1}/t_{R2})	70 µg/mL 20 µg/mL	--- ---	[14]
2	*β*-(3,4-epoxycyclohexyl)ethyltrimethoxysilane)[a]	i	~25/31 min (t_{R1}/t_{R2})	255-286 ppb	0.72-0.12	[15]
3	(*R*)-methyl benzyl isothiocyanate [b]	ii	6.57/7.12 (k_L/k_D)	0.32 µg/mL	2.34	[16]
4	(*S*)-1-(1-naphthyl) ethyl isocyanate [b]	ii	6.66/7.07 (k_L/k_D)	0.98 µg/mL	3.23	[16]
5	*N*-phthalimidyl-(*S*)-2-(6-methoxynaphth-2-yl) propionate [b]	ii	33.47/38.75 (t_{R1}/t_{R2})	0.11/0.10 pmol/mL	22.57	[17]
6	*L*-Leu-NH2 attached to DFDNB[b]	ii	13.01/14.55 (k_L/k_D)	0.002%	27.10	[18]
7	*L*-Leu-NH2 attached to CC[b]	ii	7.94/ 8.39 (k_L/k_D)	NA	6.39	[18]
8	2-(5-fluoro-2,4-dinitrophenylamino)-4-(methylthio)butanoic acid (FDNP-L-Met) [b]	ii	19.43/25.82 (t_{R1}/t_{R2})	24 pg mL^{-1}	21.69	[13]
9	2-(5-fluoro-2,4-dinitrophenylamino)-3-(phenylthio)propanoic acid (FDNP-S-benzyl-L-cysteine) [b]	ii	22.41/28.55 (t_{R1}/t_{R2})	NA	19.81	[13]
10	2-(5-fluoro-2,4-dinitrophenylamino)-3-phenylpropanoic acid (FDNP-L-Phe) [b]	ii	23.92/29.65 (t_{R1}/t_{R2})	NA	18.22	[13]
11	(R)-(+)-4-butoxy-6-chloro-N-(1-(naphthalen-1-yl)ethyl)-1,3,5-triazin-2-amine [b]		14.46/ 16.19 (k_L/k_D)	0.003%.	10.63	[19]
12	(*S*)- (–)-quinine [c]	iiia[1]	10 min (run time)	1.2-1.8 µg	4.31	[13]
		iiia[2]	09 min (run time)	1.2-1.8 µg	2.84	
		iiib	10 min (run time)	1.2-1.8 µg	1.86	
13.	(*S*)- (–)-quinine[d]	iiic	10 min (run time)	0.18 µg	1.69	[20]

CSP, chiral stationary phase; CDR, chiral derivatizing reagent; CIR, chiral inducing reagent; t_{R1} and t_{R2} are the retention time of first and second eluting isomer, respectively; k_1 and k_2, retention factor of first eluting isomer; run time, time taken by solvent system to migrate across the TLC plate; LOD, limit of detection; R_S, resolution. i, ii and iii[a^1 and a^2, b and c] represent the techniques used as direct HPLC, indirect HPLC and direct TLC [impregnating reagent (a^1; CS mixed in the slurry of silica gel before making the plates and a^2; ascending development of the plate in the solution of CS), CMPA and CIR, respectively] separation, respectively..

Table 1**:** Literature reports with present study on chromatographic separation (in terms of: R_S, LOD and resolution time) of enantiomers of SeMet using different CSPs/ CDRs/ chiral selectors/CIR.

Chiral mobile phase additives (CMPA): The CMPA approach can be carry out in TLC by mixing the chiral selector with the mobile phase system prior to development of chromatogram. Stereo selective

separation of enantiomers achieved in a system having chiral additive in the mobile phase due to one or a combination of the following 'mechanisms': Stereo selective complication in mobile phase, formation of the transient diastereomer associates between the mobile and stationary phase having different distribution properties and adsorption of the chiral reagent onto the solid stationary phase during the development of chromatogram. Enantiomeric separation of DL-SeMet has been carried out using (–)-quinine [13], as chiral mobile of additives and chromatographic data are given in Table 1.

Chiral inducing reagent (CIR): In this method chiral selector when mixed with the racemic mixture of analyte it induced the chiral environment in medium and produced transient diastereomers of analyte, therefore chiral selector here is also called chiral inducing reagent. In this method there is no requirement to produce chiral environment on the inert support of TLC for separation of enantiomers. Therefore, in this case, separation is known as achiral phase chromatographic separation and TLC is called as achiral phase TLC. This method is more advantageous over the impregnation as the required quantity of the chiral selector is very less than the quantity of the chiral selector required in impregnation methods. Literature reported the use of (–)-quinine as CIR to perform the chromatographic run, results obtained were good in terms of resolution [20].

Enantiomeric Separation by HPLC

It is most advanced and widely used method for the enantiomeric separation and also for the detection of sample. It gives better result than the TLC method in regard of resolution, accuracy and precision. There is wide choice of selection of solvents and optimization can be done easily. Highly efficient detector such as photodiode array detector (PDA) 2600 is used, which has very good detection power and make the detection of analyte molecules up to pico meter level. In this approach separation of enantiomers can be achieved directly by using chiral columns. Several columns containing chiral stationary phases, based on: amylose, cellulose, pirkle, ovomucoid, cyclodextrin, zirconia monolithic and glycoprotein have been used in recent years for enantiomeric separation and these are commercially available in market. In literature report separation of enantiomers of DL-SeMet was achieved by using following chiral stationary phase based columns; *β*-cyclodextrin [14] and *β*-(3,4-epoxycyclohexyl ethyltrimethoxysilane) [15].

Indirect approach

There are wide choices of using the solvents as mobile phase and more easily optimize the chromatographic condition in indirect method than the direct method, therefore, separation of diastereomeric pair by indirect method is sometimes easier and often better resolution obtained. This is good method for better determination of biological samples, as highly sensitive detectors are used. There are several CDRs used for derivatization of analytes and have good limit of detection. For example the Marfey's, naproxen and cyanuric chloride based chiral derivatizing reagent, because of high molar UV-Vis absorptivity (ε) and fluorescence quantum yield, shows high sensitive detector response to samples.

The rates of derivatization of enantiomers of (*R,S*)-mixture with CDRs are different, therefore derivatization conditions must be optimized. In the structure of analyte suitable reactive group should be in close proximity to the stereogenic center, which is prone, a quantitative transformation with the CDR and without forming the side products. During transformations of the enantiomeric mixture into daistereomers racemization must not be there of the CDR and as well as of the sample in the reaction.

In literature, indirect approach has been applied for separation of enantiomers of DL-SeMet by converting them into diastereomers with CDRs, owing to their separation by reverse phase HPLC. Chromatographic separation data are given (Table 1) in terms of ''resolution'' of diastereomers of DL-SeMet formed with following CDRs: (*R*)-methyl benzyl isothiocyanate (MBIC) and (*S*)-1-(1-naphthyl) ethyl isocyanate (NEIC) [16], *N*-phthalimidyl-(*S*)-2-(6-methoxynaphth-2-yl) propionate [17], CDRs based on difluorodinitrobenzene (DFDNB) and cyanuric chloride (CC) [18] based platforms and L-amino acid amides as chiral auxiliaries, DFDNB based CDRs having L-amino acids as chiral auxiliaries [13], trichloro-*s*-triazine-based new chiral derivatizing reagents having optically pure amines as chiral auxiliaries [19].

Conclusions

The liquid chromatographic techniques for separation via direct and indirect methods are very useful for qualitative and as well as quantitative analysis in pharmaceutical, synthesis and agriculture industries. Direct method is easier, less time consuming and suitable for separation of enantiomers in their pure form without any derivatization.
Direct TLC method is successful for

a. Rapid and sensitive resolution, isolation and detection of enantiomers in a range lower than the limits prescribed (1%) for pharmaceuticals in industry,
b. Less expensive in comparison to gas chromatography, HPLC and capillary electrophoresis that require highly expensive experimental set-up, running costs and more time.

Direct HPLC method is widely used and most suitable for separation of enantiomers because
a. Derivatization is not required hence it is less time consuming,
b. Most of the chiral columns having CSPs are commercially available in market,
c. Detection and isolation limit is up to pico meter level. But the chiral column is of high cost in direct method, while no requirement of chiral column in indirect method, therefore indirect HPLC method is less expensive and more useful for detection and separation of enantiomers.

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