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Enantiomeric fraction of styrene glycol as a biomarker of occupational risk exposure to styrene

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HIGHLIGHTS

- Enantioseparation of styrene glycol studied by chiral liquid chromatography.
- Enantiomeric ratio applied as biomarker for exposure to styrene.
- Achieved enantioselective resolution of 1.60 and selectivity index of 1.48
- Recovery assays show accuracy below 5% for the enantiomeric fraction.
- A faster method based on determination of anisotropy factors also developed.

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ABSTRACT

This work reports a new analytical approach for monitoring the enantiomeric ratios of styrene glycol (SG), encountered during the manufacture of plastics, by chiral liquid chromatography and the application as a biomarker for exposure to styrene. The isomers were separated using an AGP column running in the reverse phase and isocratic mode with a mobile phase consisting of 20 mM phosphate buffer saline (pH 4.15) and methanol at a flow rate of 0.8 mL/min. Photometric, polarimetric, and circular dichroism detectors were employed. The chromatographic enantioselective resolution (Rs) and selectivity index (α) values were determined to be 1.60 and 1.48 (by photometric detection), respectively. Calibration curves used for the quantification of the SG enantiomers were linear with a correlation coefficient >0.99; the detection limits were in the range of 0.03–0.16 μg, depending on the detector used. Recovery assays on synthetic samples (in triplicate) covering the full range of enantiomeric fractions (0.0–1.0) show accuracy values below 5% for the enantiomeric fraction (EF) in every case. An alternative method based on the measurement of anisotropy factors for the determination of EF, which is faster and does not require the separation of enantiomers, has also been developed. The enantiomeric excess of the toxicant biomarker styrene glycol has been determined. No previous direct enantioselective determination of styrene glycol was published.

1. Introduction

Styrene is an important intermediate used worldwide in the manufacture of plastics, synthetic rubbers, and polyester resins. In addition, it is a contaminant present in food, tobacco smoke, and engine exhaust pipes (Teixeira et al., 2008). It is metabolized through cytochrome P450-mediated oxidation to the corresponding epoxide, which is converted to diol either spontaneously or by microsomal epoxide hydrolase (Shen et al., 2009). The resulting styrene glycol (SG) has been suggested as a biomarker to study the exposure of humans to styrene.

Styrene and styrene oxide have been identified as potential carcinogenic and neurotoxic. The use of these chemicals in the manufacture of plastics and polymers and in the boat-building industry has raised concerns related to risks associated with human exposure. As styrene used in those industries can potentially migrate into foodstuffs and beverages from packing of product, humans may routinely ingest trace amounts of styrene.

The International Agency of Research on Cancer (IARC) has classified styrene oxide into group 2B, i.e., a possible human carcinogen. However, no information is available on the chronic (long-term), reproductive, developmental, or carcinogenic effects

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of styrene oxide in humans. Maternal toxicity and increased foetal mortality have been observed in rats and rabbits exposed to styrene oxide by inhalation. Several studies have reported an increased incidence of fore-stomach tumours in rats and mice exposed via gavages (experimentally placing the chemical in the stomach) (Carlson, 1998).

Styrene is not chiral, but SG posses a chiral C-atom, is optically active and thus exists as (*R*)- and (*S*)-enantiomers. Toxicity of styrene is mainly ascribable to the metabolite styrene oxide (SO) (Foureman et al., 1989), and it has been shown that a difference in toxicity between the enantiomers of SO exists (Wenker et al., 2000).

From this, because the different toxic potential of the SO enantiomers, determination of the racemic metabolites of styrene as biomarkers of styrene exposure, which is the present practice, might not truly reflect the health risk associated with exposure to styrene and a more precise measurement is the rate of both enantiomers in body fluids.

Because SO levels in blood are very low in humans, SG in blood has been suggested (Löf et al., 1986) as a bio-marker to reflect the levels of SO. In the same manner, the *R/S* ratio of SG in the blood will reflects the enantiomeric ratio of SO.

The relative proportion of enantiomers or enantiomeric fraction (EF) and its biotransformation (Zhang et al., 2014) or degradation rates in fruit juices (Ruiz-Rodriguez et al., 2015) has been recently studied in several publications. Several methods have been described for the determination of the enantiomer excess of SG by diastereoselective derivatization, including automated quantitative electrospray ionization mass spectrometry (Guo et al., 1999) detection by ^{1}H NMR (Lin and Whalen, 1994) and gas chromatography with electron capture detection and a chiral column (Wenker et al., 2001).

The diastereoselective derivatization technique presents important limitations. For example, the efficiency of the derivatization reaction shows different and unknown values for each enantiomer or racemization problems that may occur during diastereomeric synthesis (derivatization time). On the other hand, UV–vis spectroscopy is barely sensitive for the detection of SG because the simplicity of this molecule leads to low extinction coefficient. Polarimetric detection can help solve this problem if (as in this case) a high specific rotatory coefficient exists. In these cases, it is necessary to apply a method that can be used to analyse enantiomers directly, without requiring the previous derivatization step. Liquid chromatography is an excellent technique to analyse enantiomeric mixtures (Sanchez et al., 2008, 2012). In this study, this technique is applied for the first time to the enantioseparation of SG enantiomers. The use of both a universal detector ultraviolet (UV–vis) and specific chiral detectors, polarimetric (OR) and circular dichroism (CD), reinforces the reliability of the obtained results. Although several high-performance liquid chromatography (HPLC) methods for the determination of SG and its precursors frequently found in the plastic industry have been published (Yeowell-O'Connel et al., 1996; Wang et al., 2007), HPLC enantiomeric separation of SG has not been performed so far.

An attractive alternative to using chiral chromatography to determine enantiomeric ratios is based on the use of factor *g* or anisotropy factor. The *g* factor is independent of the analyte concentration, but proportional to the enantiomeric excess (EE). So far, no work has been published on the use of factor *g* as a basic parameter for measuring a biomarker of risk in the workplace. The methodology has two main advantages. One advantage is that physical separation (chromatographic) of the enantiomeric pair is not required and the other is that analysis can be conducted speedily because the detector simultaneously records absorbance (ε) and dichroic signals ($\Delta\varepsilon$), and the signal corresponding to factor *g* is directly proportional to EE or EF. In this study, the use of a chiral column working in the reversed phase mode allows precise determination of the enantiomeric ratio of SG. In addition, a fast method based on anisotropic factor measurements is developed and compared to chromatographic separation.

2. Materials and methods

2.1. Chemicals

(-)-*R*-styrene glycol ((-)-*R*-1-phenyl-1,2-ethanediol, (-)-*R*-SG, 99% purity, $[\alpha]_D^{20} = -69°$, c = 1) and (+)-*S*-styrene glycol ((+)-*S*-1-phenyl-1,2-ethanediol, (+)-*S*-SG, 99% purity from Across (Geel, Belgium), $[\alpha]_D^{18} = +66°$) were used in the study. Ethanol and n-hexane were of gradient grade and obtained from Lichrosolv Merck (Darmstadt, Germany). High purity deionised water was obtained from a Milli-Q water purification system (Millipore, Canada). Standard (-)-*R*-SG and (+)-*S*-SG solutions were prepared by dissolving 50 mg of the compounds in 50 mL of ethanol and the solutions were stored at 4 °C in the absence of light. Working solutions were prepared from these stock solutions by dilution with the appropriate mobile phase.

2.2. Standards and working standard solutions

Stock standard solutions of (-)-*R*-SG, (+)-*S*-SG, and racemic SG (36.18 mM) were prepared by dissolving the compounds in ethanol. Working standard solutions (36.18–3.6 mM) were prepared by dilution with the appropriate mobile phase. All the solvents used were of Lichrosolv gradient grade (Merck, Darmstadt, Germany). Phosphate buffer saline (PBS) was prepared from analytical reagents sodium phosphate and HCl (Merck). The solvents used as the mobile phase were filtered through 0.2-μm nylon membrane filters and degassed prior to use.

2.3. Instrumentation

The measurements were carried out on Jasco Liquid Chromatograph (Tokyo, Japan) consisting of Jasco intelligent HPLC pump model PU-1580, Degasys Populaire DP4003 (Uniflows), Jasco L-G-1580-04 quaternary gradient unit, AS-2055 Plus, and an intelligent auto sampler with a 100 μL sample loop. Three detectors, namely CD-2095 circular dichroism (CD) detector, a UV-vis detector (for simultaneous detection), and Jasco OR-2090 polarimetric detector, were used. Data acquisition, transformation, and setting of the instrument parameters were accomplished by Jasco-Borwin 1.5 software ChromConver 1.0 and Microsoft Origin.

2.4. Operating conditions

2.4.1. Chiral chromatography

A chiral AGP column (ChromTech., Congleton, UK) (150 mm × 4 mm, 5 μm particle size) consisting of α1-acid glycoprotein was used for the chromatographic separation of the enantiomers. A mobile phase consisting of 20 mM PBS at pH 4.15 and methanol (60:40, v/v) was used at a flow rate of 0.8 mL/min in the isocratic mode. The injection volume was 10 μL. Dilutions were performed using the mobile phase.

2.4.2. Anisotropy factor

The anisotropy factor was measured using Phenomenex Luna 5μ silica (25 cm × 4.6 mm, 5 μm particle size) as the stationary phase. The mobile phase was composed of hexane-ethanol (80:20 v/v) with a flow rate of 1 mL/min, and photometric and CD detectors ($\lambda_{absorbance}$ = 255 nm) were used for detection. All the