

Comparative Assessment of Methylparaben Concentration in Cosmetic Product, Analyzed Using Enzyme Biosensor and Ultra-High-Pressure Liquid Chromatography (UPLC): An Approach Towards Detection of the Environmental Toxicant with Higher Accuracy and Sensitivity

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Abstract

Methylparaben (MP) is one of the most widely used preservatives and is associated with a catalog of recently identified health hazards. Existing studies have also highlighted these compounds as environmental contaminants and toxicants that affect water quality and the associated microbial diversity of the habitat. The conventional methods for parabens detection are performed using chromatographic techniques, which assist in quantitative analysis of the group of compounds. However, the biggest challenge is the fact that such techniques are often time-consuming and costly. Furthermore, the pre-processing of samples is often required before chromatographic analysis. The growing scientific interest towards development of rapid detection techniques for the group of compounds, had brought a paradigm shift towards accepting the emerging techniques including nanotechnology and electrochemistry, for rapid detection of parabens. In the quest to explore and fabricate a point-of-detection device and develop a rapid detection method for low level quantification of MP, the current study highlights the use of a novel enzyme-based biosensor, designed by our group, to detect methylparaben concentration in one of the personal care products (PCP), commercially available in India. The comparative assessment of the Methylparaben concentration in the cosmetic sample, determined using the fabricated esterase-enzyme-based electrochemical biosensor, was observed to be more accurate than Ultra-High-Performance Liquid Chromatography (UPLC). The results indicate the applicability, accuracy, and sensitivity of the designed biosensor for quantifying methylparaben in cosmetic samples.

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INTRODUCTION

Parabens are alkyl esters of p-hydroxybenzoic acid that have been used for years as preservatives

in the pharmaceutical, food, and cosmetic industries due to their broad antimicrobial spectrum, low toxicity, and relatively low cost (Upadhyay et al., 2025) [13]. The natural occurrence of parabens has been reported in fruits such as blueberries (Sruthi N. et al., 2025) [19]. Due to their vast applications as antimicrobial preservative compounds, parabens are commonly synthesized using synthetic and enzymatic routes (Haseeb SD. et al., 2025) [17].

Different types of parabens are being chemically synthesized, which have different alkyl or aryl side chains associated with p-hydroxybenzoic acid, ranging from short chain (methyl-, ethyl-) to long chain (propyl and other higher Carbon chain length side chains) (Soni et al., 2005) [21]. The chain length-dependent antimicrobial properties of the parabens have been well explored but are restrained by associated lower solubility in water. However, due to recent studies exploring the paraben associated health hazards, the parabens are addressed as “double edged sword” (Chatterjee et al., 2024) [7]. The persistent use of packaged products containing parabens as preservatives not only increases human exposure to these pollutants but also contributes to the continuous release of these compounds into the environment, including land, rivers, and sewage treatment plant effluents (Behpour et al., 2015) [1]. As far as health hazards are concerned, existing studies have reported carcinogenic and endocrine-disrupting effects in humans and aquatic life upon exposure to parabens, thereby identifying the group of compounds as a potential threat (Hajian et al., 2015; Upadhyay et al., 2021) [5, 12]. In one of the seminal studies by Dagher et. al. (2012), the effect of the chain length of parabens was reported to be associated with cytotoxicity, analyzed using a human breast cancer cell line (Dagher et al., 2012) [4]. Due to their diverse uses and applications as preservatives in a range of products, most studies exploring the health hazards of this group of compounds have been conducted using Methylparaben (MP) (Soni et al., 2002) [11]. Methylparaben (MP), methyl 4-hydroxybenzoate, a water-soluble paraben, is one of the most used preservatives in packaged and personal care products, to improve their shelf lives (Behpour et al., 2015) [1]. It was observed that the comparative assessment of the effects of MP is also evident in the reproductive system, as shown in a study on porcine oocyte maturation (Huang et al., 2024). The toxicity of MP had also been evaluated on *Artemia franciscana*, as an aquatic model system (Comeche et al., 2017) [3]. One of the biggest challenges is the removal of parabens from various habitats, and various physicochemical techniques are often explored for this purpose, including those used in sewage treatment plants. In a recent study, *in silico* and *in vitro* esterase activity was evaluated, demonstrating the potential of pig liver esterase as a source of MP hydrolysis (Upadhyay et al., 2021) [12]. Some analytical methods have been established for the determination of MP, including high-performance liquid chromatography, Ultra-High-Pressure Liquid Chromatography, LC-MS, GC-MS, and capillary electrophoresis. Significant efforts by researchers to use electrochemical sensors for the detection of parabens have demonstrated many advantages, including high sensitivity, ease of operation, and low cost (Hajian et al., 2016; Wang et al., 2015) [6, 14]. One of the major challenges in developing biosensors is achieving specificity and sensitivity. Enzymes are explored as another biosensing element for enzyme fabrication, due to substrate specificity. The electrochemical analysis harnesses the ability to detect electron flow (current) during enzymatic catalysis.

MATERIALS AND METHODS

The current study explores the validation of a novel biosensor, designed and fabricated using pig liver esterase enzyme (published as an Indian Patent (Patent Application No. 202411052091)). The enzyme biosensor was fabricated using the previously reported method (Shen & Liu, 2007) [10], with modifications. The cosmetic sample was prepared by dissolving 0.250 grams of the selected cream sample (brand name withheld) in 4 mL of ethanol. The solution was incubated at room temperature for 20 minutes, then centrifuged at 120 rpm on a magnetic stirrer until homogeneity was achieved. The solution was diluted in 40 mL of nuclease-free water, as previously reported with modifications (Yücebaş et al., 2020) [15].

The cosmetic sample containing ca. 40 micromolar Methylparaben was analyzed by UPLC (Figure 1). The sample was separated from an Acquity UPLC column.

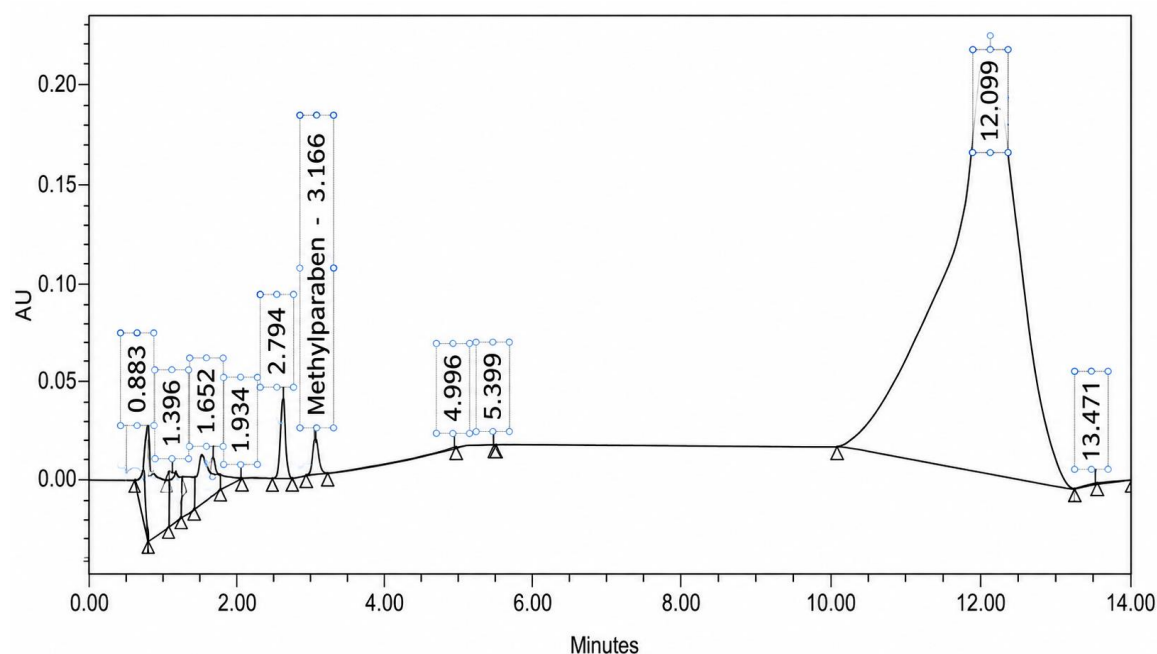


Figure 1. The UPLC analysis of the cosmetic sample. The image on the extreme left is the details on the presence of methylparaben in the selected cosmetic sample.

For UPLC quantification of Methylparaben, 0.05% TFA in water and Acetonitrile in a ratio of (90:10) v/v were used in mobile phase A, while 0.05% TFA in water and Acetonitrile in a ratio of (90:10) v/v were used in mobile phase B. The gradient method (T/percent B) was finalized as 0.0/17, 2.0/17, 4.0/33, 9.0/33, 11.0/80, 12.0/17, and 15.0/17 with wavelength detection at 222 nm, injection volume of 2 μ L, and column temperature of 40°C (Pippalla et al., 2022) [9].

RESULTS AND DISCUSSIONS

Biosensing tools and techniques are important for the rapid detection of very low levels of bioanalytes. Various analytes have been detected using catalogs of analytes with electrochemical, optical, and colorimetric biosensors (Mertens & De Maesschalck, 2015) [8]. Due to the emerging information about parabens associated health hazards and the common supplementation of MP in most of the cosmetic world, sufficient research has been done to develop rapid, selective, and sensitive methods for MP. The Cyclic Voltammetric analysis of the cosmetic sample was performed (Figure 2), and the results were compared with UPLC analysis (Table 1). The use of electrochemical sensors for detecting parabens was a pivotal outcome of the study. Amidst scarce reports, the current study highlights the role of a novel enzyme biosensor in detecting Methylparaben (MP) in cosmetic samples.

The biggest challenge in quantitatively estimating MP may be the rheological properties of the sample, which often hinder accurate detection. In the current study, a similar challenge was observed that showed variations in the quantification of MP in the cosmetic sample using two different techniques.

Results have also highlighted the pivotal role of electrochemical enzyme-based sensors in the more accurate detection of the compound than UPLC. The chromatographic assessment of Methylparaben is well documented (Pippalla et al., 2022) [9]. However, the role of viscosity mediated variation in the detection of Methylparabens had not been explored. The study may be further extended to enable rapid, relatively low-cost quantification of Parabens in environmental samples, which need to be evaluated for their toxicant potential and may be pivotal in preventing associated health hazards.

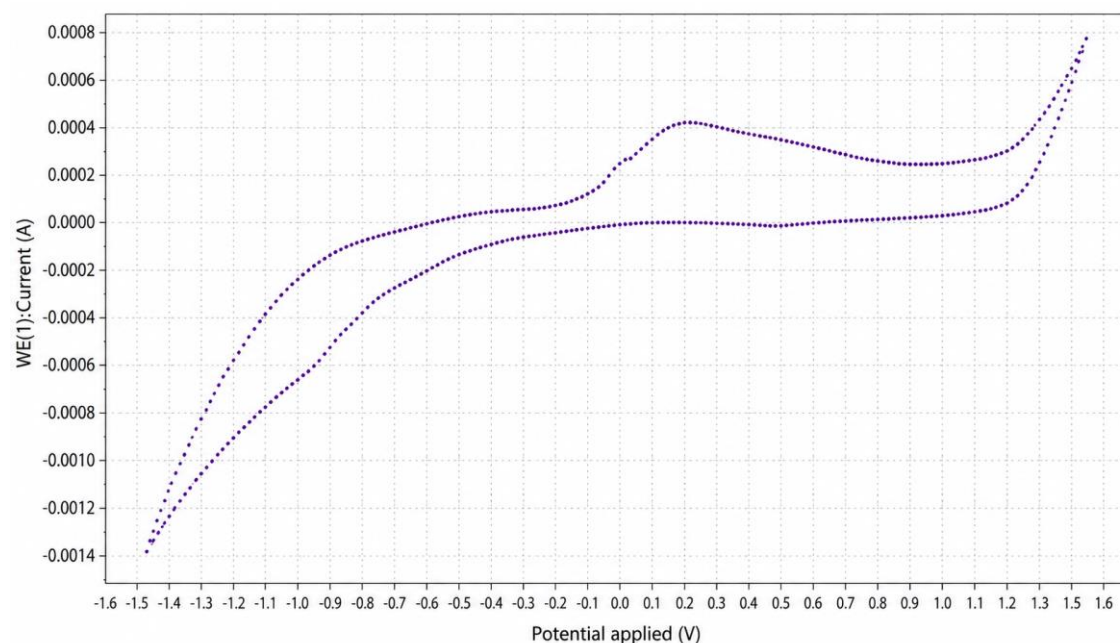


Figure 2. Cyclic Voltammetric (CV) analysis of cosmetic sample on enzyme-based biosensors. The CV was performed using an electrochemical workstation (Metrohm India Pvt. Ltd., India).

Table 1. Concentration of methylparaben analyzed by UPLC and Prepared enzyme biosensor.

Detection Technique	Concentration of methylparaben present in cosmetic sample (X)
UPLC	11 micromolar.
Enzyme Biosensor	34 micromolar.
Concentration reported by the manufacturer (ca.)	40 micromolar.

CONCLUSION

Based on the results, it may be concluded that an enzyme-based electrochemical biosensor is a more reliable technique than the conventional chromatographic method for detecting Methylparaben in a viscous solution. The study also provided impetus for the use of enzyme-based sensors for quality assessment and low-level detection of Methylparaben.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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