

UNIVERSITI TEKNOLOGI MARA

**PERFORMANCE EVALUATION OF
A NEW MICROEXTRACTION
METHOD USING NATURAL DEEP
EUTECTIC SOLVENTS FOR THE
QUANTITATIVE DETERMINATION
OF STEROID DRUGS**

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ABSTRACT

The misuse of anabolic steroids and their increased pharmaceutical use have led to drug residues polluting surface waters, posing risks to public health. Detecting these compounds in natural waters requires sensitive analytical methods. Traditional extraction methods like liquid-liquid extraction (LLE) and solid-phase extraction (SPE) use large amounts of toxic organic solvents, which not aligned green chemistry principles. This study aims to develop natural deep eutectic solvents (NADES) as eco-friendly alternatives with adjustable properties and strong extraction ability to improve efficiency, selectivity, and sustainability. Hence, this study focuses on the development and application of NADES as green alternatives for the analytical determination of anabolic steroid drugs. The research is structured into four main phases; preparation and characterization of NADES; evaluation of NADES as a dispersive solvent in vortex-assisted dispersive liquid-liquid microextraction (VADLLME); computational studies to confirm the stability of selected NADES by theoretical and experimental study from previous objective; and evaluation of Alginate-NADES (Alg-NADES) solid sorbent beads in dispersive micro-solid phase extraction (D- μ -SPE) for the analysis of selected anabolic steroid drugs, alongside a greenness assessment using the AGREE metric calculator. The physicochemical characterization of Lactic acid-Sorbitol ((LA)-Sor) was conducted using ATR-FTIR, confirming its successful preparation. The (LA)-Sor solvent was then applied in VADLLME prior to chromatographic analysis (HPLC-DAD) for the determination of two anabolic steroids, testosterone and nandrolone in tap water and food supplements. The optimized VADLLME parameters were as follows: (LA)-Sor at a molar ratio of 2:1, a 500 μ L NADES volume, and 200 μ L of chloroform as the extraction solvent. The method exhibited good linearity within the range of 0.5–10 mg L^{-1} for testosterone and nandrolone ($R^2 = 0.9990$ – 0.9996), with satisfactory intraday (1.79–11.57%) and interday (3.24–19.26%) precision, low detection limits (0.0019–0.0117 mg L^{-1}), and acceptable relative recovery (88.63–114.21%). The NADES's stability was confirmed by DFT and experimental study at molar ratio of (LA)₂-Sor. To further enhance the performance of NADES, it was combined with sodium alginate to form solid sorbents (Alg-NADES) beads, which were characterized using ATR-FTIR, FESEM, BET, and TGA prior to their application in the D- μ -SPE method. A Plackett-Burman Design (PBD) screening and Response Surface Methodology, Box-Behnken Design (RSM-BBD) were employed to optimize key analytical parameters, including agitation speed, salt addition, extraction time, and desorption solvent volume, all of which demonstrated high agreement (95% confidence) with model predictions. The optimized D- μ -SPE conditions yielded a linear range of 1–10 mg L^{-1} ($R^2 = 0.9991$ – 0.9999), LOD values between 0.1280–0.2986 mg L^{-1} , and accuracy within 92.73–117.78%. The method exhibited adequate precision, with intraday and interday RSDs ranging between 1.59–8.16% and 1.91–9.37%, respectively. VADLLME and D- μ -SPE methods yielded a score of 0.6 and 0.5 in Analytical Greenness Calculator (AGREE) respectively, indicating an adequate alignment with the 12 Principles of Green Analytical Chemistry (GAC).

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CHAPTER 1

INTRODUCTION

1.1 Research Background

During the Covid-19 pandemic, restrictions on outdoor activities led many people to seek alternative ways to maintain their health at home, including exercising via online platforms, building home gyms, or consuming substances like anabolic steroids, which are typically prescribed for hormone deficiencies or muscle loss in AIDS patients (Kernas, 2019). The misuse of these steroids (testosterone as main androgens), especially among athletes and bodybuilders seeking enhanced performance, has long been an issue, with about 57% of world-class amateur athletes admitting to using performance-enhancing drugs (Kernas, 2019) as presented in Figure 1.1.

These synthetic steroidal pharmaceuticals have been reported to pose some of the highest ecological and health risks (Yang et al., 2021). According to Gunnarsson et al. (2019), these compounds can significantly disrupt the endocrine system of intact organisms, leading to adverse physiological and behavioural outcomes, a concern further emphasized by Ojogoro et al. (2021). The increasing use of such pharmaceuticals also raises environmental concerns, as drug residues are often excreted unchanged or in active metabolite forms through various biochemical pathways, contaminating water sources at low yet potentially harmful concentrations (Rivera-Utrilla et al., 2013; Molnár et al., 2022). This contamination poses significant challenges for detection and removal, contributing to public health concerns due to unknown long-term effects of trace pharmaceutical exposure. In response, drug testing technologies have advanced to detect long-term steroid metabolites at lower thresholds, though drug developers continually modify substances to evade detection. To ensure fairness and accuracy, labs must store baseline steroid data and account for individual biological variations, but these comprehensive tests remain costly, labour-intensive, and time-consuming due to complex procedures such as sample preparation (National Institute on Drug Abuse, 2018).