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Method validation for the detection of undeclared Furosemide drug contaminations in herbal weight loss supplements using LC-MS/MS

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Numerous herbal weight loss supplements are widely consumed globally; however, there is a general concern regarding undeclared synthetic substances. Furosemide (4-chloro-2-(furan-2-ylmethylamino)-5-sulfamoylbenzoic acid) is a potent diuretic substance, and it is prohibited for athletes due its ability to mask the presence of performance enhancing drugs in urine and its ability for rapid weight loss by excreted water as urine. This study aims to validate a new method for the detection of undeclared furosemide in weight loss supplements available in the local market using LC-MS/MS. In developing the method validation protocol, after testing the recovery and the matrix effect for suitability, two different herbal weight loss supplements were pulverised and mixed to make the blank matrix. Then 100.0 mg of the mixture was introduced to a centrifuge tube and 10.0 mL acetonitrile (LC/MS grade) was added to it, and sonicated for 10 minutes. The resultant solution was centrifuged for 10 minutes at 5000 rpm and the supernatant was double diluted and filtered using nylon filters (0.22µm). Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction was used for coloured extractants. Seventeen (17) commercially available weight loss supplements were purchased from local retailers and analysed for the presence of furosemide using the developed method. In the developed method, a 1:1 ACN:Water mobile phase, biphenyl column operating at 40°C, 0.2 sec loop time was used. Under these conditions, the retention time of furosemide is 3.82 minutes. The developed method was validated according to the ICH (M10 2022) and Eurachem guidelines (second edition 2014). The linearity (r^2 = 0.99) of the calibration plot, selectivity, LOD (0.14 nmol m^{-3}) and LOQ (0.48 nmol m^{-3}), trueness (±15%), precision (±5%), recovery (70.96%-81.06%) and matrix effects (103.0%-107.1%) were determined to ensure the method's validity for the intended purpose. None of the supplements contained detectable furosemide levels using the validated LC/MS/MS method. The absence of detectable furosemide in the analysed samples suggests that the tested supplements may not be adulterated with this specific diuretic or it is below the detection limit of this method.

Keywords: Furosemide, LC/MS/MS, Method development, Method validation, Weight loss supplements