

# **Validation of HPLC-DAD method for analysis of sibutramine, fluoxetine, caffeine, theobromine and phenolphthalein as potential adulterants in weight loss dietary supplements**

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## **Introduction**

Weight loss dietary supplements are dynamically growing group of consumer's products as they are used for speeding up the process of weight loss in the treatment of obesity. In order to comply with the minimal regulatory requirements in demonstrating efficacy (Koncz et al., 2021), some manufacturers are economically motivated for the addition of unauthorized ingredients to enhance their slimming effects. This substandard practice known as adulteration is becoming health concern and more important since dietary supplements are not assessed and approved prior to commercialization (Rocha et al., 2016). Moreover, these products are widely available in pharmacies, some retail stores or over the Internet (Rebiere et al., 2012). Therefore, detection of the substances used for adulteration remains a great challenge for the regulatory. The aim of this study was to validate a HPLC-DAD method for simultaneous determination of sibutramine, fluoxetine, caffeine, theobromine and phenolphthalein, which are reported as the most common adulterants of weight loss supplements (Khazan, 2014).

## **Materials and methods**

Nineteen weight loss supplements sampled from the market were tested for eventual presence of sibutramine, fluoxetine, caffeine, theobromine and phenolphthalein. Standard solutions were prepared at concentration of 0.2 mg/mL using reference substances. To prepare sample solutions, homogenized powders obtained by direct emptying of capsules or grounding the tablets were sonicated with a mixture of phosphate buffer pH 2.5 and acetonitrile in ratio 64:36 (v/v). This mixture was used as a mobile phase delivered at a flow rate of 1 mL/min. The extracts were filtered through a 0.45 µm pore size membrane filter and injected at a volume of 10 µL on a HPLC system Agilent 1200 (Agilent Technologies, USA) equipped with a LiChrospher® 60 RP-select B (125 mm × 4 mm, 5.0 µm) column at 40 °C. The detection wavelength was 225 nm (Zhivikj et al., 2021).

## **Results and discussion**

The method was fully validated according to the ICH Q2 guideline (ICH, 2005). Specificity of the method was confirmed from the chromatograms

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obtained from the blanc, individual standards, mixture of the standard solutions and placebo containing the most commonly used excipients. The chromatograms showed no interfering peaks with the peak of any studied compound. The resolution factor for the analytes peaks was found to be more than 2 from the nearest eluting peak and with no interference appeared in the retention of analytes (confirmed by analysis of peak purity), indicating on the satisfactory specificity and selectivity of the method. The linearity of the method was tested using seven concentrations (0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2 and 0.3 mg/mL) of the standard solutions and evaluated using correlation coefficient ( $R^2$ ) obtained from regression analysis.  $R^2$  for the calibration curves for all of the components was consistently  $\geq 0.999$ . The accuracy of the method was determined from the recovery study by adding known amounts of each compound corresponding to 50, 100 and 150% of the respective working concentration. The method is accurate (98.17-100.26%) and precise ( $RSD \leq 5\%$ ). The robustness of the method was tested for the various factors such as flow rate ( $\pm 0.1$  mL/min), column temperature ( $\pm 5$  °C), pH of mobile phase ( $\pm 0.1$ ), mobile phase ratio ( $\pm 2\%$ ) and concentration of phosphate in the buffer ( $\pm 0.01$  mol/L). The system suitability parameters obtained for each compound were evaluated and showed no significant changes indicating that the method is robust at small, but deliberate modifications.

The method was used for screening of the presence of adulterating substances in nineteen weight loss supplements sampled from the market. Undeclared substances were detected in three of the tested products: fluoxetine was detected in two products and theobromine was detected in one of the products.

## Conclusion

A simple, fast and convenient HPLC-DAD method was adopted for simultaneous separation and assay of five potential adulterants in weight loss dietary supplements available in Macedonian market (sibutramine, fluoxetine, caffeine, theobromine and phenolphthalein). The proposed method is specific, selective, linear, accurate, precise and robust; therefore, it is valid for detection of five adulterants

potentially present in weight loss supplements in analysis with a 10-min run time. The major challenge and at the same time an advantage of this method is the possibility for simultaneous detection and identification of adulterants due to their different physicochemical properties and distinct polarities, as well as difficulty related to the different matrices of weight loss supplements.

This method was successfully applied to local marketed products and hence can be useful for the routine analysis of any of the five investigated compounds illegally added in weight loss supplements.

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