

## Undeclared furosemide in food supplements.

Stanislava Ivanova\*, Kalin Ivanov

Department of Pharmacognosy and Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University of Plovdiv, Plovdiv, Bulgaria

### Abstract

Recent studies claim that dietary supplements could contain pharmaco-active ingredients or undeclared drug substances with a potential health risk. Most often these products are contaminated with anabolic steroids, ephedrine, sibutramine, diuretics and others. The intake of a contaminated dietary supplement could lead to serious health consequences (the pharmacological effect of the substances, side effects, drug interactions and many others) or other negative consequences like failure of a doping test (for professional athletes). We have performed HPLC screening for furosemide. Measurements were performed at 228 nm at a flow rate 0,8 ml/min. The mobile phase was composed of methanol and water in a ratio 80:20 (v/v). The pH of the mobile phase was 3,3 adjusted with Phosphorous acid. Chromatographic column - Microsorb-MV 100-5 C18 150 × 4.6 mm. We have analysed 30 food supplements in the category "prostate health". We have found undeclared furosemide in 10% of the samples.

**Keywords:** Food supplements, Dietary supplements, HPLC furosemide, Undeclared substances, Doping.

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### Introduction

In the last two decades dietary supplement industry has grown fast. Nowadays it is considered as a multi-billion dollar industry [1]. The main factors for the fast grow of that industry could be summarised as: liberal regulation worldwide (dietary supplements are categorised as food in most countries), lack of consistent requirements for analytical control (purity testing, quantity and quality of the active constituents and others), fast introduction of new products, high consumption of dietary supplements, aggressive advertising campaigns. In most countries in the world it is not necessary to obtain approval before producing/selling dietary supplements [2]. In these countries there are notification based systems for introduction of new dietary supplements [3]. The notification approach costs minimal premarket resources compared to the registration approach. In some countries like Argentina, Brazil, Canada there are registration-based systems [3]. The registration based approach requires a more lengthy review. The review process includes analysis of a large number of data (details on the specifications of the finished product; label, evidence supporting the safety; certificates of analysis, other certificates (good manufacturing practice). The registration based approach is much better in assuring product safety and quality but most countries use notification-based systems.

In the same time the consumption of dietary supplements grows fast: consumers want to improve or preserve physiologic or metabolic functions. The consumers' expectations range from disease/condition prophylaxis to disease/condition

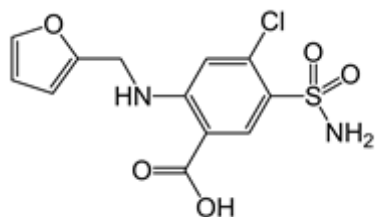
treatment and even cure [3]. Worldwide dietary supplements should not claim to diagnose, treat, cure, or prevent any disease [2] because they refer to the products intended to compensate for the nutrients that otherwise might not be sufficiently present in the diet [3]. However a recent research of 443 websites showed alarming results: 81% of the food supplements made health claims. 55% claimed the product treated, prevented, diagnosed, or cured specific diseases [2]. Such products could mislead consumers and that could lead to serious health consequences: inadequate self-healing, underestimation of health condition and others.

As a result of the lack of obligatory analytical control and liberal regulation in most parts of the world, Recent studies claim that dietary supplements often contain pharmaco-active ingredients or undeclared drug substances with a potential health risk [4-18]. Most often dietary supplements are contaminated with anabolic steroids, ephedrine, sibutramine, diuretics and others. Contaminations can occur accidentally (because of poor manufacturing practices) or intentionally [2]. The intake of a contaminated dietary supplement could lead to serious health consequences (the pharmacological effect of the substances, side effects, drug interactions and many others) or other negative consequences like failure of a doping test (for professional athletes). Analysis of the data from US emergency rooms for a ten year period estimated 23,000 visits due to adverse effects after intake of food supplements. 2156 of these people were hospitalised [2].

In most cases of contamination, the products refer to the following categories dietary supplements: weight loss, dietary

supplements for energy, and dietary supplements for muscle building. In this study we decide to analyse another category of dietary supplements - prostate health. We have performed series of analysis for screening of furosemide.

The discovery of the first diuretics close on sixty years ago remains a major milestone in therapeutic progress. Furosemide (4-Chloro-2-[(furan-2-ylmethyl)amino]-5-sulfamoylbenzoic acid) is a loop diuretic which was patented in 1959. It was approved for medical use in 1964. Furosemide (Figure 1) is used in the treatment of congestive heart failure and edema [19]. The diuretic effect of orally administered furosemide appears usually 30 minutes after intake [19]. Furosemide may also be given intravenously at 40 to 80 mg/day. Nowadays it is one of the most widely used diuretic in clinical practice. The World Anti-Doping agency considers furosemide as a doping substance because its intake may mask the intake of other doping substances. The substance of furosemide is cheap. In the United States generic medicines containing furosemide cost about US\$0.15 per day in Bulgaria (European Union) a generic oral drug containing furosemide costs about 0.035 Euros per day. The price of the substance and its effect are possible reason for adulteration of dietary supplements with it [16,17].



**Figure 1.** Structure of furosemide.

Because of its great clinical importance, numerous analytical procedures has been developed for detection of furosemide in pharmaceutical products and biological samples [20-26].

## Materials and Methods

### Materials

**Reference standard:** Furosemide (Sigma-Aldrich).

**Reagents:** Methanol for HPLC (Sigma-Aldrich); Phosphorous acid-(Sigma-Aldrich); Deionised water.

**Samples:** 30 food supplements with beneficial effects on prostate health. Samples were purchased from dietary supplement stores, pharmacies, and internet.

### Methods

HPLC/UV detection

**Instrumentation:** Varian Pro Star HPLC system, UV detector, chromatographic column- Microsorb-MV 100-5 C18 150 × 4.6 mm, Hamilton syringes designed for use with manual HPLC injection ports (25 ml).

**Chromatographic conditions:** The mobile phase is composed of methanol and water in a ratio 80 : 20 (v/v). pH- 3,3 adjusted with Phosphorous acid (Sigma-Aldrich).

**Elution:** Isocratic elution. Measurements were performed at 228 nm at a flow rate 0,8 ml/min. with an injection volume of 20 microliters.

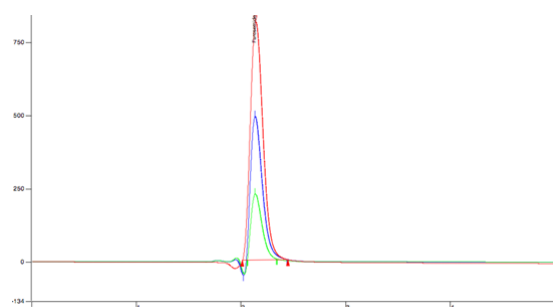
**Preparation of standard solution and test solutions:** Stock solutions were prepared in methanol–water (v/v 7:3) at different concentrations. The standard solution and test solutions were prepared by dilution in solvent containing methanol 70% and water 30%.

## Results and Discussion

### Validation of the method

The method was validated according to ICH guidelines [27]. For the screening we have used Varian Pro Star HPLC system: chromatographic column- Microsorb-MV 100-5 C18 150 × 4.6 mm and UV detector. The detector was set at 228 nm. We have prepared a mobile phase composed of methanol and water in a ratio 80 : 20 (v/v). The pH of the mobile phase was adjusted to pH- 3,3 by adding Phosphorous acid (Sigma-Aldrich).

Standard solution of furosemide: 5.0 mg of the referent substance furosemide (Sigma Aldrich) was weighed in a volumetric flask (10 ml). The substance was dissolved in 10 ml solution containing (70% methanol/30% water). Concentration of the stock solution- 500 µg/ml. Furosemide working solutions were prepared by dilution of the stock solution. We have established the retention time for furosemide standard - 2.19 min. Figure 2 represents chromatogram of a standard solution of furosemide.



**Figure 2.** Chromatograms of standart solutions of furosemide standart in different concentrations.

**Linearity:** A typical calibration curve (Figure 3) of furosemide peak area within a concentration range of 250 µg/ml - 1,5 µg/ml was obtained with the following linear regression line:  $Y=125904x+366243$  ( $R^2=0.9865$ ).

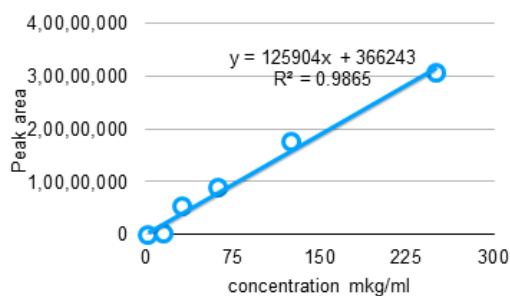


Figure 3. Calibration curve.

**Detection limit:**

$DL = 3.3 \sigma/S$

Where:  $\sigma$ =the standard deviation of the response; S=the slope of the calibration curve; DL= detection limit.

The slope S was estimated from the calibration curve of the analyte.

We have established  $DL = 0.031$  mkg/ml

**Precision:** We have evaluated the intraday precision (repeatability) and inter-day precision (intermediate precision). The relative standard deviation values, in all cases, were less than 1.4% for repeatability and intermediate precision. These results show that the method has adequate precision in determination of furosemide.

**Accuracy:** The accuracy was evaluated by replicating the analysis of the samples from three different concentrations 50, 75, and 100%. For each concentration level, we have prepared three tests. Mean recovery:  $98.46 \pm 0.14\%$  respectively. The results show that the method is accurate.

**Robustness:** We have evaluated the influence of variations of pH in the mobile phase and its composition (proportion of methanol/water). We have established that pH of the mobile phase could vary between 3,2 and 3,5 with insignificant impacts on the peak areas, peak asymmetry and the retention time. We have established that the proportion of methanol/water in the mobile phase could vary with  $\pm 1\%$  with insignificant impacts on the peak areas, peak asymmetry and the retention time. Each experiment was performed in replicate (n=6).

**Application of the method in analysis of dietary supplements**

This study is focused on food supplements in the category - “prostate health”. Analysed samples were purchased from dietary supplement stores, pharmacies and internet. All food supplements were in the category prostate health. We divided the samples in two categories: saw palmeto containing food supplements (n=11) and others (n=19). The labels of selected food supplements were observed about - the statement of identity, nutrition labelling, ingredient labelling, and nutrient content. The supplements from the second group were labelled

that contain more than one “active” ingredient. All samples had plant origin. Most of them were labelled as “natural”. Sample preparation included dilution with water/methanol (3/7 v/v). 30 working test solution were prepared by dilution of the test stock solutions. All test solutions were filtrated twice. The first step included prefiltration - to remove larger particles from the solutions. After that it was performed subsequent, finer filtration. We used syringe filters (pore size 0.25  $\mu$ m). All samples were introduced in the chromatographic system through manual injector port. We used Hamilton Syringes for HPLC (25 mcl.). Injection- 20 mcl of each sample. We found that 3 of the analysed samples contained undeclared furosemide. Figures 4, 5 and 6 present chromatograms of the furosemide - positive samples.

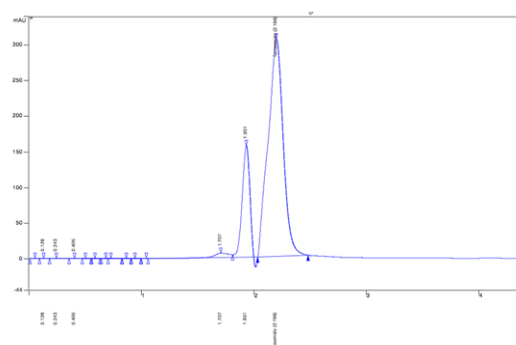


Figure 4. Chromatogram of sample N14.

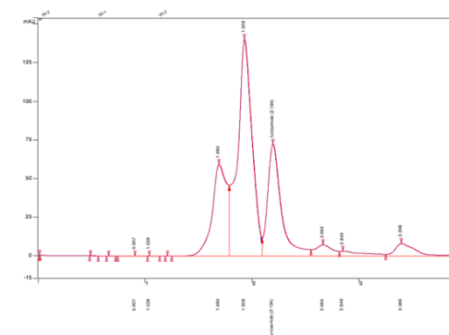


Figure 5. Chromatogram of sample N 18.

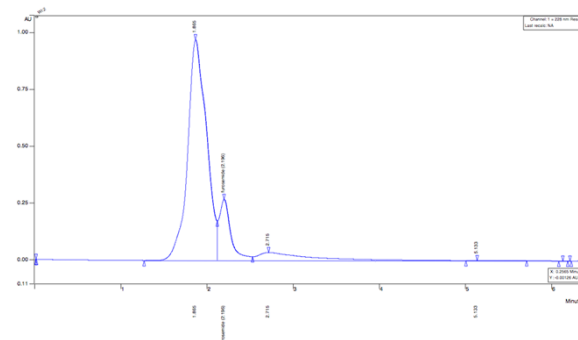


Figure 6. Chromatogram of sample N 26.

The undeclared furosemide varied between 9 and 35, 5 mg/per capsule. All contaminated food supplements were labelled: 1 capsule 2 times daily. The intake of undeclared furosemide in

these doses would definitely lead up to a significant increase in diuresis of the patient. For some consumers that might be a positive effect. Another effect would be a significant lowering in the blood pressure that could be very dangerous for some consumers. The intake of furosemide is associated with many side effects. Common side effects of furosemide intake include: hypokalaemia, hypomagnesaemia, dry mouth, headache and others. The intake without knowing of furosemide could lead also to many dangerous drug interactions. Another great risk of the intake of undeclared furosemide is a positive doping test. If a professional athlete uses a furosemide-contaminated food supplement he would give a positive doping sample. Furosemide is considered as a doping substance. It is included in the category-diuretics and masking agents. The presence of undeclared furosemide in food supplements is extremely dangerous.

## Conclusion

The use of dietary supplements all over the world is steadily growing. This fact raises some important concerns about the safety and efficacy. Many researchers claim that some dietary supplements could contain pharmaco-active ingredients or undeclared drug substances with a potential health risk. We have performed HPLC analysis of 30 different dietary supplements in category "prostate health". The present HPLC method provides satisfactory outcomes with low level of detection, good accuracy and robustness. The method application allowed the determination of furosemide with accurate results. We have found undeclared furosemide in 10% of the samples. Poor manufacturing processes or intentional contamination with drug or other pharmaco-active substances continue to occur in dietary supplements. The intake of a contaminated dietary supplement could lead to very serious health consequences.

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**\*Correspondence to**

Stanislava Ivanova  
Department of Pharmacognosy and Pharmaceutical Chemistry  
Medical University of Plovdiv  
Plovdiv  
Bulgaria