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Research Article

Development and validation of HPLC-PDA method for simultaneous determination of sibutramine, furosemide, and phenolphthalein in solid form weight loss products

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Abstract

The illegal adulteration of weight loss products using pharmaceutical substances such as sibutramine, furosemide, and phenolphthalein poses a serious threat to public health. This study aimed to develop and validate a method for the simultaneous quantification of these compounds in weight loss dietary supplements using high-performance liquid chromatography with photodiode array detection (HPLC-PDA). The optimized method employed an InertSUSTAIN Phenyl column (250 mm × 4.6 mm, 5 µm), with a mobile phase of acetonitrile and 0.5% phosphoric acid (45% : 55%), a flow rate of 1 mL/min, and detection at 225 nm. Samples were extracted using 96% ethanol with a simple preparation procedure. The method was validated according to AOAC guidelines, including selectivity, linearity, accuracy, precision, limits of detection (LOD), limits of quantification (LOQ), and dilution integrity. The method showed excellent linearity ($R^2 > 0.998$), acceptable recoveries (95–103%), and low LODs and LOQs (0.161–0.758 µg/mL). Application of the method to 19 commercial weight loss products revealed 4 samples containing sibutramine and 2 samples containing phenolphthalein. This method is suitable for routine use in food control laboratories to ensure the safety of weight loss products.

Keywords: *sibutramine, phenolphthalein, furosemide, HPLC-PDA, weight loss products.*

1. INTRODUCTION

In recent decades, overweight and obesity have emerged as serious global public health concerns. It is estimated that the economic burden attributable to overweight and obesity accounted for approximately 2.19% of the global GDP in 2019 and could rise to 3.29% by 2060 without effective interventions [1]. Obesity not only increases the risk of chronic diseases such as cardiovascular disease, diabetes, and cancer but also negatively impacts quality of life and life expectancy [2].

Driven by the increasing demand for weight loss, many consumers have turned to natural-origin slimming products or health supplements. However, some of these products have been illegal adulterated with pharmaceutical substances such as sibutramine, furosemide, and phenolphthalein to enhance their weight-reducing effects. Sibutramine, a serotonin-norepinephrine reuptake inhibitor, was previously approved for the treatment of obesity but has been banned due to its association with hypertension and cardiovascular side effects. Phenolphthalein, formerly used as a laxative, has been withdrawn from pharmaceutical use due to its potential carcinogenicity. Furosemide is an effective loop-type diuretic that can cause dehydration and electrolyte imbalance if misused [3–5].

In Vietnam, according to Circular No. 10/2021/TT-BYT, furosemide is included in the list of substances prohibited for use in the production and trading of dietary supplements. This Circular also references Appendix V – "List of drugs and pharmaceutical ingredients banned from importation and production" – issued together

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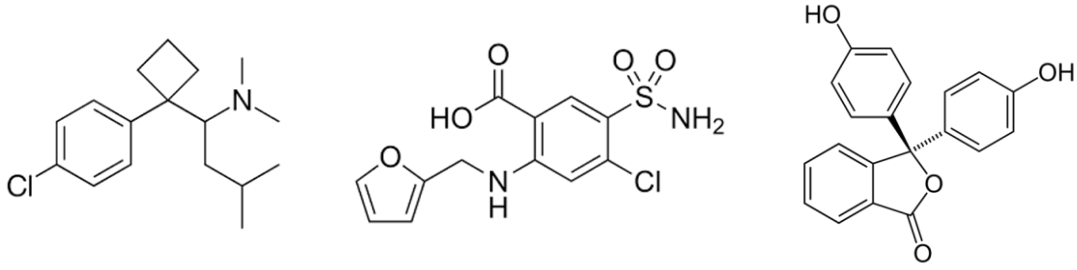
with Decree No. 54/2017/ND-CP, to classify sibutramine and phenolphthalein as similarly prohibited substances for use in dietary supplement products. Therefore, all three substances — furosemide, sibutramine, and phenolphthalein — are banned for use in the manufacturing and trading of dietary supplements in Vietnam [6, 7].

Currently, various analytical methods have been developed to detect these substances in dietary supplement products, including thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) coupled with UV, photodiode array (PDA) detectors, or mass spectrometry (LC-MS/MS) [8–13]. Among these, LC-MS/MS offers high sensitivity and selectivity; however, it involves high equipment and operational costs and requires highly skilled personnel. TLC is a low-cost technique but has limited accuracy and sensitivity. HPLC-PDA represents a balanced approach in terms of accuracy, sensitivity, and practical applicability, making it suitable for use under the current instrumentation conditions available at Drug Quality Control Centers.

Several domestic and international studies have applied HPLC-PDA for the quantification of sibutramine, furosemide, and phenolphthalein in weight loss products. However, most of these methods employed phosphate or acetate-buffered mobile phases, gradient elution, long analysis times, and complex sample preparation procedures [8–10]. Therefore, the development of a simultaneous analytical method for sibutramine, furosemide, and phenolphthalein that offers high accuracy, is easy to implement, and is compatible with the current instrumentation available in quality control laboratories is essential. Such a method would contribute to improving the efficiency of monitoring the quality of weight loss supplements, ensuring consumer safety, and supporting regulatory authorities in food safety management.

The objective this study is to develop and validate an HPLC-PDA method for the simultaneous quantification of sibutramine, furosemide, and phenolphthalein (**Figure 1**) in weight loss dietary supplement products, in a manner compatible with the practical conditions of quality control laboratories.



Sibutramine *Furosemide* *Phenolphthalein*

Figure 1. *Chemical structures of sibutramine, furosemide, and phenolphthalein*

2. MATERIALS AND METHOD

2.1. Study subjects and samples

The subjects in this study include three pharmaceutical compounds: sibutramine hydrochloride (HCl), furosemide, and phenolphthalein, which are substances at risk of being illegally adulterated in weight loss supplements.

According to Decree No. 15/2018/ND-CP, weight loss dietary supplements available on the market come in various dosage forms, including capsules, tablets, granules, powders, and solutions [14]. In this study, we initially developed and validated the method using solid-form placebo matrices, which represent the most commonly distributed forms such as instant coffee, tea bags, tablets, and capsules, prior to expanding its application to other dosage forms. The placebo matrix was prepared from a mixture of herbal ingredients and common excipients typically found in powdered weight loss products on the market, including: celery powder (15%), green tea powder (15%), Crataegus (hawthorn) (10%), Jasminum subtriplinerve (10%), lotus leaf (10%), and lactose (40%).

Test samples were commercial weight loss products labeled with the term “weight loss” and were randomly collected from Binh Duong from June to August 2024.

To optimize the preparation procedure, a total of 100 mg of placebo matrix was weighed into a 15 mL centrifuge tube, followed by the accurate addition of 100 µL of a mixed standard stock solution containing the three analytes at a concentration of 500 µg/mL, and 4.9 mL of extraction solvent. The mixture was vortexed for 1 minute, then ultrasonicated for 10 minutes, allowed to cool to room temperature, and vortexed again for 30 seconds. After settling (or centrifugation if necessary), the supernatant was filtered through a 0.45 µm membrane filter. The final analyte concentration in the extract was approximately 10 µg/mL.

The spike sample for validaiton was prepared as the following procedure: An amount of 100 mg of the placebo matrix was weighed into a 15 mL centrifuge tube. A precisely measured volume of a mixed standard stock solution was added, containing sibutramine at 100 µg/mL, and phenolphthalein and furosemide at 50 µg/mL, corresponding to three spiking levels: limit of quantification (LOQ), approximately 5× LOQ, and mid-calibration range. The volume was brought up to 5 mL with extraction solvent, followed by vortexing for 1 minute, ultrasonic extraction for 10 minutes, cooling to room temperature, and vortexing again for 30 seconds. After settling (or centrifugation, if necessary), the supernatant was filtered through a 0.45 µm membrane filter.

2.2. Chemicals and standards

Standard substances: Sibutramine hydrochloride (Lot G1457682, 93.37%) and phenolphthalein (Lot G1430033, 99.9%) were obtained from LGC (UK). Furosemide (Lot QT129 060121, 99.7%) was supplied by the Ho Chi Minh City Drug Quality Control Institute (Vietnam).

Chemicals and solvents: Acetonitrile (HPLC grade), methanol, 96% ethanol, distilled water, glacial acetic acid, and 85% phosphoric acid (analytical grade) were used.

Note: All chemicals and solvents used for mobile phase preparation were filtered through 0.45 µm membrane filters and degassed prior to use.

2.3. Equipment

A Hitachi Chromaster CM5000 high-performance liquid chromatography (HPLC) system was used for chromatographic analysis. Analytical balances included a Mettler Toledo balance (5-decimal precision) and a Precisa balance (4-decimal precision). Other laboratory equipment included a vortex mixer, a centrifuge, and an Elma ultrasonic bath.

2.4. Research method

2.4.1. Optimization of chromatographic conditions

During method development, several chromatographic parameters were kept constant throughout the experiments: column oven temperature at 30°C, flow rate of 1.0 mL/min, and injection volume of 10 µL.

To determine the optimal chromatographic conditions for the simultaneous separation of sibutramine HCl, furosemide, and phenolphthalein, the following main parameters were investigated:

Detection wavelength: The UV absorption spectra of each analyte were recorded to select a common detection wavelength that provides strong absorbance for all three compounds while minimizing matrix interference. The selected wavelength offered clear, stable signals suitable for simultaneous quantitative analysis.

Stationary phase: Restek Ultra C18 (250 mm × 4.6 mm, 5 µm) and InertSUSTAIN Phenyl (250 mm × 4.6 mm, 5 µm) were chosen to evaluate base on the criteria such as resolution between peaks, reasonable retention time, and acceptable peak asymmetry (tailing factor).

Mobile phase: Several solvent systems were tested to optimize separation performance. The following mobile phases were investigated:

For the Restek Ultra C18 column: Acetonitrile – Water (45% : 55%); Acetonitrile – 0.5% Phosphoric acid (45% : 55%); Acetonitrile – 20 mM Ammonium acetate buffer, pH 3.5 (45% : 55%); Gradient elution with mobile phases A (acetonitrile) and B (20 mM ammonium acetate buffer, pH 3.5): 0–1 min: 80% B, 3 min: 60% B, 11–12 min: 50% B and 13–17 min: 80% B.

For the InertSUSTAIN Phenyl column: Acetonitrile – 0.5% Phosphoric acid (45% : 55%), Acetonitrile – 0.5% Phosphoric acid (39% : 61%).

Column oven temperature: In addition to the default temperature of 30°C, a higher temperature of 40°C was also tested to assess its impact on peak resolution and peak shape, allowing for the selection of the optimal temperature for analysis.

2.4.2. Evaluation of Sample Preparation Conditions

Ultrasonic-assisted extraction was employed as the sample preparation method. The study investigated the extraction efficiency of various solvents, including: water**,** methanol**,** 50% methanol (v/v)**,** 96% ethanol, and 1% acetic acid. Solvent selection was based on the following criteria: extraction efficiency, impurity in chromatographic (i.e. absence of matrix inteferences) and laboratory safety considerations.

2.4.3. Method validation

The method was validated according to the AOAC Appendix K guidelines [15], covering the following parameters:

Selectivity: Ensured by the absence of analyte peaks in solvent and matrix blanks. Placebo samples spiked with standards must exhibit peaks at corresponding retention times as in standard chromatograms, with matching UV spectra.

System suitability: Assessed through parameters including repeatability (injection precision), resolution (Rs), tailing factor (Tf), and theoretical plate number (N).

Linearity: Calibration curves were constructed at multiple concentration levels and evaluated using the correlation coefficient (R^2).

Accuracy (Recovery): Performed by spiking placebo matrix at three concentration levels and calculating percentage recoveries.

Precision: Includes repeatability (intra-day precision) and intermediate precision (inter-day and analyst-to-analyst variability).

Limit of Detection (LOD) and Limit of Quantitation (LOQ): Determined based on signal-to-noise ratios, with S/N $\geq$ 3 for LOD and S/N $\geq$ 10 for LOQ.

Dilution integrity: Evaluated by assessing recovery and precision after dilution of spiked test samples.

2.4.4. Application of the method to samples

After successful validation, the method was applied to analyze actual weight loss dietary supplement products available on the market. Qualitative analysis was performed by comparing the retention times and UV spectra of the peaks in the test solution chromatograms with those of the standards. If a peak appeared within ± 2.5% of the retention time of sibutramine, furosemide, or phenolphthalein in the standard chromatogram, the corresponding UV spectrum was examined for confirmation. If confirmed, the analyte content was quantified. Quantitative analysis was based on the following formula: X (mg/g) = (C_x×5×D)/(m). Where: C_x: analyte concentration (mg/mL) calculated from the calibration curve {C_x = (y – intercept)/slope}, D: dilution factor (if applicable, used when peak area exceeds the calibration range), m is sample weight (g).

The obtained results were used to assess the feasibility and practical applicability of the method for routine quality control of weight loss dietary supplements.

2.4.5. Data processing method

Microsoft Excel 365 was employed.

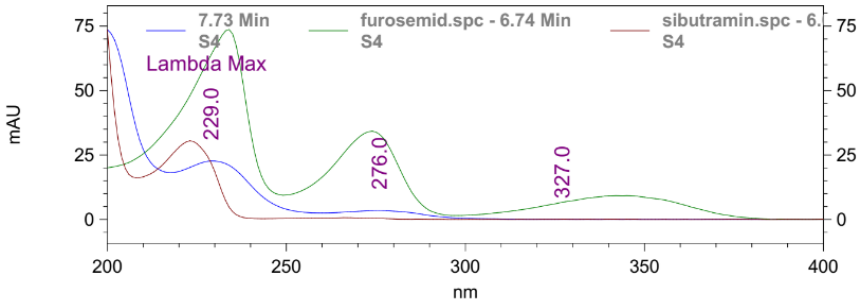
3. RESULTS AND DISCUSSION

3.1. Investigation of chromatographic conditions

3.1.1. Selection of detection wavelength

The UV absorption spectra of the three analytes in methanol, recorded in the range of 200–400 nm using a DAD detector, are presented in **Figure 2**. The UV absorption spectra of the three analytes (sibutramine, furosemide, and phenolphthalein) recorded in the range of 200–400 nm showed that all three compounds exhibited strong absorption in the short-wavelength UV region. Specifically, furosemide displayed absorption maxima at approximately 234 nm and 274 nm, sibutramine at around 223 nm, while phenolphthalein exhibited a broad spectrum with a clear signal in the 220–230 nm region. After overlaying the spectra and evaluating

signal intensities in the absorption regions, 225 nm was selected as the common detection wavelength. This region provides strong absorbance signals for all three compounds, especially furosemide, which has the highest absorbance intensity at this wavelength. The choice of 225 nm ensures adequate sensitivity, selectivity, and methodological simplicity for routine implementation in quality control laboratories, despite the capability of the PDA detector to monitor multiple wavelengths simultaneously.



Overlaid spectra

Figure 2. *UV absorption spectra of sibutramine, furosemide, and phenolphthalein in methanol (200–400 nm)*

This selection is also consistent with previous studies employing HPLC-PDA for the quantification of sibutramine and phenolphthalein, in which 225 nm was frequently chosen due to its suitability for detecting aromatic compounds and conjugated functional groups [3, 4].

3.1.2. Investigation of Chromatographic Stationary Phase, Mobile Phase, and Column Oven Temperature

The results of the investigation of the stationary phase, mobile phase, and column oven temperature are presented in **Figure 3** at Acetonitrile – 0.5% phosphoric acid (45% : 55%), InertSUSTAIN Phenyl column, column oven temperature 30℃.

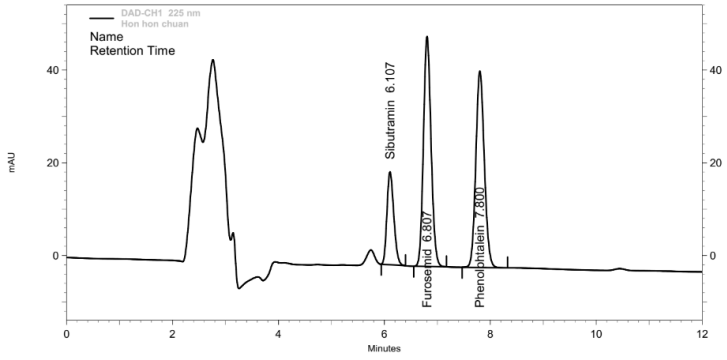


Figure 3. *Chromatogram under the selected chromatographic conditions*

During the chromatographic condition optimization, the Restek Ultra C18 column (250 mm × 4.6 mm, 5 µm) was initially used with various mobile phase systems. When using the acetonitrile–water (45% : 55%) system, the furosemide peak eluted early—even before the solvent peak—and no sibutramine peak was observed throughout the 30-minute analysis. Replacing the mobile phase with acetonitrile–0.5% phosphoric acid (45% : 55%), all three analyte peaks appeared close to each other, but the asymmetry factor of the sibutramine HCl peak exceeded 2.0, which did not meet the requirements for peak shape. In addition, although other mobile phase systems were tested, such as acetonitrile–ammonium acetate buffer based on literature [5] and gradient elution on the same Restek C18 column, the results still failed to meet the criteria for resolution, retention time, and peak symmetry.

The study then proceeded with the InertSUSTAIN Phenyl column (250 mm × 4.6 mm, 5 µm) using acetonitrile–0.5% phosphoric acid as the mobile phase. At a 45:55 ratio, the analyte peaks were clearly separated, with resolution values greater than 2.0, asymmetry factors below 1.5, and a short analysis time.

However, reducing the acetonitrile content to 39% resulted in poorer resolution between the sibutramine and furosemide peaks, which no longer met the criteria. Additionally, when column oven temperature was increased to 40°C, resolution between these peaks again dropped below acceptable limits. Therefore, the condition using the InertSUSTAIN Phenyl column with acetonitrile–0.5% phosphoric acid (45% : 55%) was selected as optimal for simultaneous analysis of the three compounds.

Selected chromatographic conditions:

- Mobile phase: Acetonitrile – 0.5% Phosphoric acid (45% : 55%)
- Column: InertSUSTAIN Phenyl, 250 mm × 4.6 mm, 5 µm
- Column oven temperature: 30°C
- Flow rate: 1 mL/min
- Detection wavelength: 225 nm
- Injection volume: 10 µL

3.2. Investigation of sample preparation conditions

The fixed conditions during the investigation included: 100 mg of sample matrix and 5 mL of extraction solvent.

The results of the extraction solvent evaluation are presented in **Figure 4.**

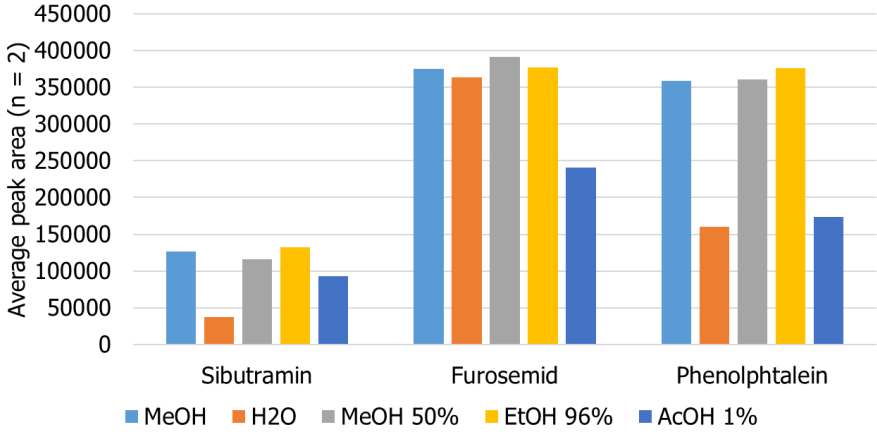


Figure 4. *Results of Extraction Solvent Evaluation*

To select a suitable extraction solvent for the simultaneous analysis of sibutramine, furosemide, and phenolphthalein, five solvents were evaluated: methanol, water, 50% methanol, 96% ethanol, and 1% acetic acid. The results showed that both methanol and 96% ethanol produced high peak areas for all three analytes. Among them, 96% ethanol provided comparable or higher signal responses than methanol, with lighter-colored extract solutions and fewer colored impurities. In contrast, water and 1% acetic acid yielded low extraction efficiency, especially for sibutramine and phenolphthalein. Meanwhile, 50% methanol gave intermediate results but showed no significant advantage. Therefore, 96% ethanol was selected as the optimal extraction solvent due to its high extraction efficiency, cleaner chromatograms, and greater safety in laboratory conditions.

The selected sample preparation procedure is as follows:

Standard solution: Dissolve appropriate amounts of sibutramine HCl, furosemide, and phenolphthalein standards to obtain a mixed standard solution with concentrations of approximately 10 µg/mL, 5 µg/mL, and 5 µg/mL, respectively, in methanol.

Sample solution: Accurately weigh about 100 mg of the test sample into a 15 mL centrifuge tube. Add exactly 5 mL of 96% ethanol, vortex for 1 minute, sonicate for 10 minutes, let cool, then vortex for 30 seconds. Allow to settle or centrifuge if necessary. Filter the supernatant through a 0.45 µm membrane filter.

3.3. Method validation

3.3.1. Selectivity

The selectivity assessment results shown in **Figure 5** indicate that no interfering peaks were observed at the retention times corresponding to sibutramine, furosemide, and phenolphthalein on the chromatograms of the methanol blank and placebo samples. The retention times of sibutramine, furosemide, and phenolphthalein in the placebo spiked samples matched those in the standard solution chromatogram. The UV spectra of the placebo spiked samples were consistent with those of the standard solutions (similarity coefficient > 0.99).

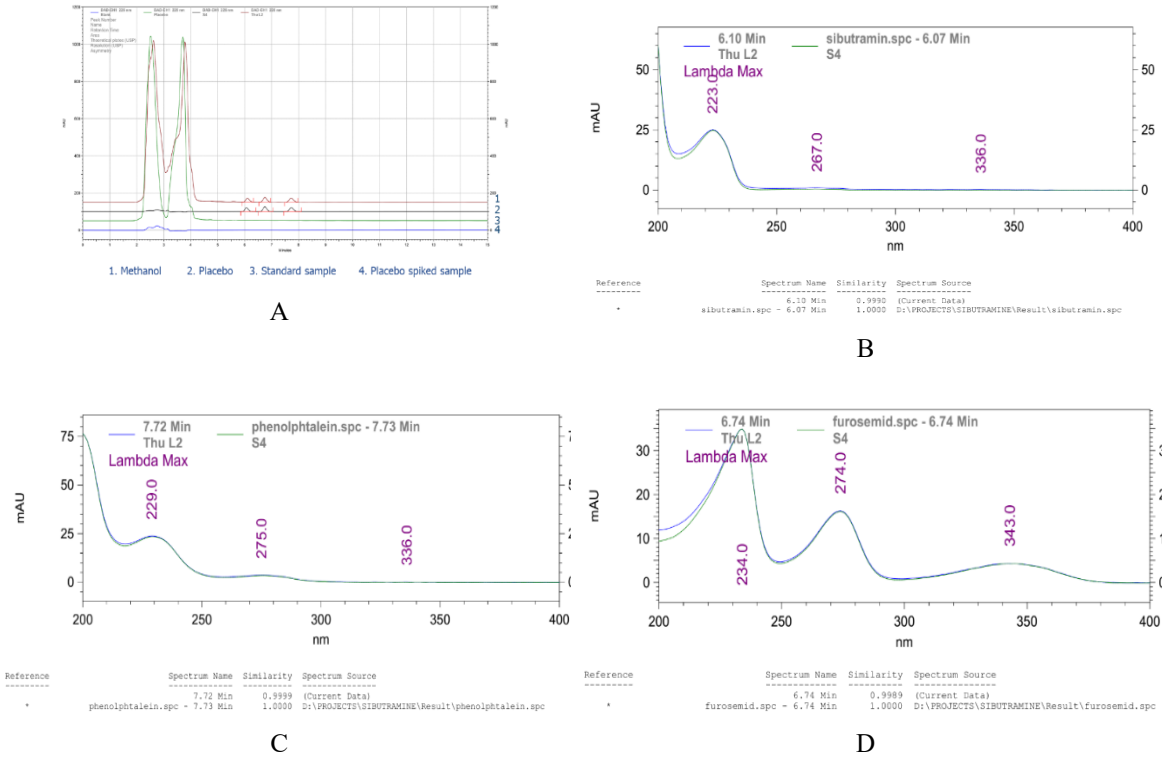


Figure 5. *Results of method selectivity validation.*
(A) Overlay chromatograms for specificity validation. (B) Overlay of UV spectra of sibutramine. (C) Overlay of UV spectra of phenolphthalein. (D) Overlay of UV spectra of furosemide

3.3.2. System suitability

The system suitability was assessed through six replicate injections of the standard solution. The results are shown in **Table 1.**

Table 1. *Results of system suitability evaluation*

Analyte	Theoretical plates (N)	Tailing factors (As)	RSD% of peak area	Resolution (Rs)
Sibutramine HCl	10097	1.253	0.24	-
Furosemide	10149	1.122	0.27	2.697
Phenolphthalein	10824	1.100	0.29	3.491

The %RSD of six replicate injections of the standard solution for all three analytes was below 2.0%; the resolution values were all greater than 2.0; tailing factors ranged from 0.8 to 2.0; and the number of theoretical plates exceeded 2,000. The method for simultaneous quantification of the three compounds demonstrates acceptable system suitability.

3.3.3. Linearity

The calibration curve was constructed using standard solutions, consisting of 7 mixed standard solutions at concentrations within the linear range, each injected three times. The results are presented in **Table 2.**

Table 2. *Results of linearity validation of the method*

Analyte	**Concentration range** (µg/ml)	**Correlation coefficient** ($R^2 \geq 0{,}995$)	**Regression equation**	**Bias value**
Sibutramine HCl	0.758 – 18.954	0.9988	$\hat{y}$= 76055.8x	-11.4% ÷ +0.1%
Furosemide	0.404 – 10.102	0.9987	$\hat{y}$ = 198434.9x	-12.1% ÷ -0.3%
Phenolphthalein	0.404 – 10.090	0.9987	$\hat{y}$ = 178693.4x	-11.8% ÷ -0.1%

Remark: When analyzing the significance of the regression equations using Microsoft Excel, the correlation coefficients (R^2) for all three analytes were greater than 0.995, indicating a high degree of correlation. The analytical method demonstrated good linearity over the studied concentration range.

3.3.4. Accuracy

Accuracy was evaluated using placebo matrix samples spiked with the analytes at three concentration levels, with six independent preparations at each level. The results are presented in **Table 3.**

Table 3. *Accuracy assessment results of the method*

Analyte	**Concentration**	**Mean recovery (%) ± SD** (n = 6)
Sibutramine HCl	LOQ (0.76 ppm)	99.10 ± 1.81
	~5 × LOQ (4.2 ppm)	100.52 ± 0.27
	Mid-point of the calibration curve (9.5 ppm)	100.34 ± 1.78
Furosemide	LOQ (0.4 ppm)	99.78 ± 0.58
	~5 × LOQ (2.2 ppm)	98.68 ± 0.31
	Mid-point of the calibration curve (5.1 ppm)	95.06 ± 1.66
Phenolphthalein	LOQ (0.4 ppm)	99.60 ± 0.58
	~5 × LOQ (2.2 ppm)	102.96 ± 0.24
	Mid-point of the calibration curve (5.1 ppm)	102.84 ± 1.47

Remarks: All three analytes at the three concentration levels exhibited recoveries within the acceptable range: 85%–110% at LOQ level, and 90%–108% at the other two levels, in accordance with Appendix K – AOAC guidelines.

3.3.5. Precision

Precision includes repeatability and intermediate precision. Repeatability was evaluated using placebo samples spiked at the mid-point concentration of the calibration curve (as shown in **Table 3**). Intermediate precision was assessed under the same procedure as repeatability, but with variation in conditions, such as different days and different analysts. The results of precision validation are presented in **Table 4.**

Table 4. *Results of method precision validation*

Analytes	Day 1 RSD% (n=6)	Day 2 RSD% (n=6)	Combined RSD% (n=12)
Sibutramine HCl	1.78	0.82	2.07
Furosemide	1.75	0.34	1.67
Phenolphthalein	1.47	0.35	2.07

Remarks: The intra-day RSD (n = 6) was below 3%, and the inter-day RSD (n = 12) was below 4% for all three analytes, meeting the requirements of AOAC Appendix K. Therefore, the method is considered to have acceptable repeatability and intermediate precision.

3.3.6. Limit of detection (LOD) and limit of quantification (LOQ)

The LOD corresponds to the analyte concentration that yields a signal-to-noise ratio (S/N) of at least 3, and the LOQ corresponds to the concentration that yields an S/N of at least 10, with acceptable accuracy and precision. Results are shown in **Table 5.**

Table 5. *Limit of detection and limit of quantitation of the method*

			Sibutramine HCl	***Furosemide***	***Phenolphthalein***
S/N ratio values	Standard solution	*Injection 1*	2.824	3.275	2.645
		Injection 2	42.294	47.751	37.174
		Injection 3	87.467	94.545	77.427
	Simulated test sample	*Injection 1*	24.717	34.857	25.298
		Injection 2	20.825	30.499	22.66
		Injection 3	13.506	20.537	15.598
Limit of Detection (LOD) of the Method (µg/mL)			0.303	0.161	0.161
Limit of Detection (LOD) of the Method in Sample Matrix (µg/g)			15.163	8.072	8.072
Limit of Quantitation (LOQ) of the method (µg/mL)			0.758	0.404	0.404
Limit of Quantitation (LOQ) of the method in the sample matrix (µg/g)			37.908	20.180	20.180

Remarks: The analytes exhibited relatively low limits of quantitation, which are suitable for analysis using the HPLC-PDA technique.

3.3.7. Effects of dilution

The effect of dilution was evaluated by preparing placebo samples spiked with standards at a concentration five times higher than the highest point on the calibration curve. The samples were then diluted 10-folds using 96% ethanol, and recovery and repeatability were assessed. The results are presented in **Table 6.**

Table 6. *Validation results for the effect of dilution (n = 3)*

Analytes	**Sibutramine HCl**	**Furosemide**	**Phenolphthalein**
Mean recovery ± SD (%)	100.93 ± 0.56	99.71 ± 0.34	100.93 ± 0.81
RSD%	0.55	0.34	0.80

Remarks: The average recoveries fall within the acceptable range of 90.0–108.0%, and RSD values are ≤ 3.0%, which meet the AOAC requirements. Therefore, a 10-fold dilution of the test solution does not affect the analytical results.

Currently, there are numerous studies focusing on the individual or simultaneous quantification of sibutramine, furosemide, and phenolphthalein in food matrices using various techniques such as thin-layer chromatography (TLC), HPLC-PDA, and liquid chromatography-tandem mass spectrometry (LC-MS/MS). The study conducted by Panadda Phattanawasin et al. employed a TLC technique combined with image analysis (TLC-image analysis). This method is notable for its simplicity, speed, and very low cost, requiring only basic equipment and simple reagents. It is an ideal choice for preliminary screening of large numbers of samples or for laboratories with limited instrumental capacity. However, a major drawback of TLC is its relatively low sensitivity and accuracy compared to high-performance liquid chromatography (HPLC) methods. The quantification capability of TLC is typically approximate (634 ng/spot for sibutramine in this study) and often insufficient for accurately quantifying low concentrations or resolving structurally similar compounds in complex sample matrices [13]. Compared to TLC-based studies, the method developed in this research offers higher accuracy, greater sensitivity, and broader applicability for drug quality control laboratories. Most studies using HPLC-PDA applied C18 reversed-phase columns with mobile phases containing phosphate or acetate buffers and employed gradient elution, as summarized in **Table 7**. In contrast, this study developed a method using a phenyl stationary phase column that met all validation requirements, featured a short analysis time (retention time of approximately 8 minutes), and used a simple isocratic mobile phase consisting of only acetonitrile and 0.5% phosphoric acid. Ethanol 96% was used as the extraction solvent, offering a less toxic alternative to methanol, and the sample preparation procedure was simple and cost-effective, thus enhancing the method's practicality. Although C18 columns are more commonly available in provincial drug quality control laboratories, the choice of a phenyl column in this study was based on several considerations. The phenyl column provided better separation of sibutramine, phenolphthalein, and furosemide from each other and from matrix interferences in complex food samples, resulting in improved peak shapes and higher resolution during preliminary trials compared to the C18 column. Moreover, the use of a different stationary phase such as phenyl contributes to expanding technical options for laboratories, particularly when dealing with complex separation challenges where the C18 column may not be optimal. This highlights the flexibility and adaptability of chromatographic methods to the specific characteristics of analytes and sample matrices. Other studies by Truong Van Than et al. and Duong Thi Mai Hoa et al. employed LC-MS/MS techniques. These are advanced and highly sensitive analytical technologies that offer superior selectivity. For example, the study by Duong Thi Mai Hoa et al. demonstrated the ability to quantify sibutramine and its derivatives (N-desmethyl sibutramine and N-didesmethyl sibutramine) at very low concentrations (LOQs ranging from 0.15 to 0.30 µg/kg), while the study by Truong Van Than et al. achieved an LOQ of 30 µg/kg for sibutramine [11, 12]. This high sensitivity is ideal for detecting illegally adulterated active substances even when present at trace levels in complex food matrices. However, a key limitation of LC-MS/MS methods is the requirement for expensive instrumentation, high operation and maintenance costs, and the need for highly skilled personnel. These factors hinder the widespread implementation of LC-MS/MS in laboratories with limited resources, especially at provincial-level drug testing centers in Vietnam. Additionally, sample preparation for LC-MS/MS often involves multiple steps to thoroughly clean the samples and protect the mass spectrometer, particularly the ion source, from contamination, which could affect system stability. On the other hand, illicit substances added to food generally need to be present at levels sufficient to exert pharmacological effects. Therefore, the method developed in this study ensures adequate sensitivity to detect and accurately quantify such compounds

Table 7. *Comparison of domestic studies using HPLC with the present study*

Authors (Year)	Analyte	Sample preparation and chromatographic conditions	LOQ	Application results
Panadda Phattanawasin et al. (2012)	Sibutramine	Silica gel 60 F254 TLC plate, mobile phase: toluene–n-hexane–diethylamine (9:1:0.3), detection using Dragendorff's reagent.	634 ng/spot	6 out of 20 samples contained sibutramine.

Cao Cong Khanh, Nguyen Tuong Vy, Hoang Quynh Trang (2013)	Sibutramine, furosemide, dexamethasone, piroxicam	Extraction with methanol. C18 column, detection at 225 nm, flow rate 1.0 mL/min. Mobile phase: methanol and 0.05 M ammonium acetate buffer (pH 3.5), gradient elution for 15 minutes, injection volume 20 µL.	Furosemide: 0.2 ppm Sibutramine, dexamethasone, piroxicam: 0.4 ppm	5 out of 20 samples contained sibutramine, ranging from 9.65 to 26.1 mg/capsule.
Le Dinh Chi, Le Thi Hong Hao (2015)	Sibutramine	Extraction with methanol, purification with n-hexane. C18 column, 30ºC, detection at 225 nm, flow rate 1.0 mL/min. Mobile phase: acetonitrile and 0.05 M KH_2PO_4 buffer (pH 4.0), gradient elution for 17 minutes, injection volume 50 µL.	Sibutramine: 0.5 ppm	11 out of 34 capsule samples contained sibutramine, ranging from 22.0 to 69.2 mg/g.
Truong Van Than et al. (2019)	Sibutramine, phenolphthalein	Sample processed using QuEChERS. Reconstituted in methanol–water (1:1). C18 column, gradient elution with water and acetonitrile containing 0.1% formic acid.	30 µg/kg	No sibutramine or phenolphthalein detected in 46 samples.
Duong Thi Mai Hoa et al. (2020)	Sibutramine, *N*-desmethyl sibutramine, *N*-didesmethyl sibutramine	Extraction with methanol, cleanup using activated carbon. C18 column, gradient mobile phase of acetonitrile and 2 mM ammonium acetate + 0.1% formic acid.	Sibutramine: 0.15 µg/kg; N-desmethyl and N-didesmethyl sibutramine: 0.30 µg/kg	6 out of 30 samples contained sibutramine (0.817–31.4 mg/g); 4 samples contained N-desmethyl sibutramine (0.27–3.10 mg/g).
Nguyen Thai Ngoc Mai, Nguyen Thi Ngoc Van (2023)	Sibutramine, phenolphthalein	Extraction with methanol, cleanup using n-hexane. C18 column, 30ºC, detection at 225 nm, flow rate 1.0 mL/min. Mobile phase: 0.1% formic acid in acetonitrile, methanol, and 0.2% ammonium acetate buffer, gradient elution for 19 minutes, injection volume 20 µL.	Sibutramine, phenolphthalein: 1 ppm	8 out of 10 samples contained sibutramine (17.51–14,460.69 mg/kg); 4 samples contained phenolphthalein (21.07–16,349.46 mg/kg).
This study (2024)	Sibutramine, furosemide, phenolphthalein	Extraction with 96% ethanol. Phenyl column, 30ºC, detection at 225 nm, flow rate 1.0 mL/min. Isocratic mobile phase: acetonitrile – 0.5% phosphoric acid (45:55), injection volume 10 µL.	Sibutramine: 0.76 ppm; Phenolphthalein, furosemide: 0.40 ppm	4 out of 19 samples contained sibutramine (0.51–41.24 mg/g); 2 samples contained phenolphthalein (0.12–9.13 mg/g).

3.4. Application of the method to samples

Based on the validated procedure, several food supplement samples collected from the market were analyzed. The results are presented in **Table 8** and **Figure 6.**

Table 8. *Analysis results of selected market products ("–": not detected)*

STT	Samples	Content (mg/g)		
		Sibutramine HCl	Furosemide	Phenolphthalein
01	Hard capsule 01 (powder content)	41.24	-	-
02	Instant coffee 01	0.51	-	0.12
03	Instant coffee 02	-	-	-
04	Instant coffee 03	-	-	-
05	Hard capsule 02 (capsule shell)	-	-	-
06	Hard capsule 03	40.76	-	9.13
07	Instant coffee 04	-	-	-
08	Tablet 01	-	-	-
09	Hard capsule 04	16.40	-	-
10	Tea bag 01	-	-	-
11	Tea bag 02	-	-	-
12	Cereal powder 01	-	-	-
13	Protein powder	-	-	-
14	Hard capsule 05	-	-	-
15	Hard capsule 06	-	-	-
16	Tablet 02	-	-	-
17	Hard capsule 07	-	-	-
18	Effervescent tablet 01	-	-	-
19	Tea bag 03	-	-	-

Remarks: Among the 19 food supplement samples surveyed on the market, 4 samples were found to contain sibutramine and 2 samples contained phenolphthalein, while furosemide was not detected in any sample. Notably, all samples containing phenolphthalein also concurrently contained sibutramine. Samples containing sibutramine hydrochloride in the range of 16.40 to 41.24 mg/g were all in hard capsule dosage form (with an average powder weight of approximately 0.5 g per capsule). This is scientifically consistent with the previously approved therapeutic dosage of sibutramine, which started at 10 mg/day and could be increased up to 15 mg/day. In the case of instant coffee containing sibutramine at a lower concentration (0.51 mg/g), the smallest dosing unit was a 16 g sachet, which could still deliver a pharmacologically relevant dose. Phenolphthalein, previously used as a laxative at doses ranging from 10 to 20 mg/day, was detected at 9.13 mg/g in a hard capsule sample and 0.12 mg/g in an instant coffee sample—both concentrations aligning with expected pharmacological dosing. For capsule samples found to contain both sibutramine and phenolphthalein, dilution of the test solution was necessary to ensure concentrations fell within the validated working range of the analytical method. These findings highlight the continued prevalence of illegal adulteration with banned substances in weight-loss food products. The HPLC-PDA method has proven to be an effective and reliable analytical approach for the detection and quantification of such adulterants.

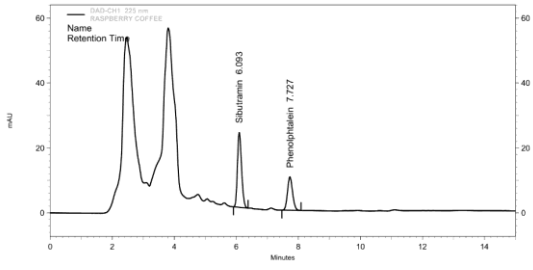


Figure 6. *Chromatogram of the instant coffee 01*

4. CONCLUSIONS

The study successfully developed and validated a method for the simultaneous quantification of sibutramine, furosemide, and phenolphthalein—illicitly added substances in weight-loss food products. The method is well-suited to the practical conditions of routine laboratory analysis, offering time- and cost-efficiency, simple operation, and achieving acceptable accuracy, precision, along with low limits of detection (LOD) and limits of quantification (LOQ). Within the scope of the study, 19 food samples were analyzed, among which 4 samples were found to contain sibutramine and 2 samples contained phenolphthalein. To expand this research, further validation should be conducted on other sample matrices such as semi-solid and liquid forms, as well as on sibutramine derivatives and other pharmaceutical compounds potentially adulterated into food products. The use of LC-MS/MS technology could enhance sensitivity and improve the detection capability for analytes at lower concentrations. Regulatory authorities are urged to strengthen the monitoring and quality control of weight-loss food products on the market to ensure consumer safety.

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