

Research Article



A Fully Validated HPLC-UV method for Quantitative and Qualitative Determination of Six Adulterant Drugs in Natural Slimming Dietary Supplements

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Accepted on: 31-08-2014; Finalized on: 31-10-2014.

ABSTRACT

A fully validated HPLC-UV method for identification and quantification of pharmaceutical preparation containing molecules frequently founded in illegal slimming products (caffeine-furosemide-phenolphthalein-sibutramine-fluoxetine and orlistat) has been developed. The proposed method uses a Hypersil BDS C18 (4.6×250,5μ) with a gradient using an ammonium acetate buffer pH=5 as aqueous phase and acetonitrile as organic modifier. The obtained method was fully validated based on its measurements (accuracy-linearity-precision-intermediate precision-LOD-LOQ-and trueness). A HPLC-UV method was obtained for the identification and quantification of this kind of pharmaceutical preparation or dietary supplement, which will reduce analysis time and quantity of solvent.

Keywords: Adulterated, Dietary supplements, HPLC-UV, Validation.

INTRODUCTION

Many of natural health products (NHP) have a history of safe use and are increasingly used for health care purpose¹, one of these purposes is weight loss. But some products marketed or represented as NHP for reduces weight adulterated with drugs.¹⁻¹⁶ Products adulterated contain substances that are not declared on the label, including prescription medications or other potentially dangerous ingredients .if people use one of these products, they will be exposed to the added drugs or substances without their knowledge which may present serious risks to their health.

The adulteration of health products that are promoted as natural but contain prescription drugs or its derivatives have become worldwide problem, especially those promoted for weight loss.⁷⁻¹²

Internationally, the use of illegal weight-loss medication and dietary supplements has led to many cases of serious health damage and occasionally even to death.^{7,8,14}

Such products can be easily purchased as dietary supplements or medical products in some pharmacies, drug stores, retail stores as well as in beauty salons or over the internet.^{1,12}

After a risk analysis based on the international studies, reports and the side effects that present on the patients who take dietary supplements for weight loss,6 substances that cause weight loss, most frequently founded in illegal products, have been selected. These substances belong to different pharmacological categories: Anorectics (Sibutramine, it has structural similar to amphetamines and is serotonin-nor adrenaline reuptake inhibitors SNRI^{6,9}) used to reduce appetite¹⁶, it banned by EMA⁵and withdrawn from marketed in 2010.^{7,12} Stimulants (Caffeine, used to induce temporary

in either mental or physical function and it can be either natural or synthetic origin, the FDA classify moderate intake of caffeine as generally recognized as safe, 200-300 mg per day).¹⁵

Antidepressant (Fluoxetine, used to alleviate anxiety¹, it tasted for weight loss but is not approved for the treatment of obesity¹⁷). Laxative (phenolphthalein, used to raise intestinal transit, in 1990 FDA changed its classification in OTC drugs to not generally recognized as safe.¹² Diuretics^{1,3} (Furosemide, used to increase loss of water¹ also not approved for slimming purposes) and Orlistat a selective pancreatic and gastrointestinal lipase inhibitors, an only OTC slimming drug approved by FDA for long-term use.^{4,17}

MATERIALS AND METHODS

Chemicals, reagents and samples

Caffeine, furosemide, phenolphthalein, fluoxetine, sibutramine, orlistat were kindly obtained from National Institute for the Control of Pharmaceuticals and Biological Products in Syria, the purity of all those standards is known and greater than 97% (w/w). Acetonitrile from Sigma Aldrich were HPLC grade. Methanol from Merck were HPLC grade. Ammonium acetate and phosphoric acid were analytical grade from SHAM lab and water was ultra-pure HPLC grade.

Chromatographic conditions

The method was developed on HPLC-UV with Ezchrom Elite software and BDS Hypersil (C18, 4.6×250, 5μm) at 50°C.

The mobile phase was composed of (A) ammonium acetate 0.025Mm solution (1.925g/l) adjusted to PH 5 with phosphoric acid and (B) acetonitrile. A gradient was applied from 20% (v/v) to 100% of mobile phase (B).The



mobile phase was delivered at a flow rate 1.5ml/min. Samples were stored at 4°C prior to the injection. The injection volume was 20µl. The detection wavelength was 220nm. The chromatographic conditions show at table 1.

Stock solution

Stock solution of caffeine, furoseamide, phenolphthalein, fluoxetine, sibutramine and orlistat were prepared at a concentration of 100µg/ml separately by dissolving the appropriate amounts of the reference standards in methanol.

For system suitability test, a standard mixture of the six medicines at 50µg/ml was prepared in methanol.

Table 1: Chromatographic conditions

Column	C18 (4.6×250, 5µm)		
Mobile phase A	Ammonium acetate 0.025mM PH=5 buffer		
Mobile phase B	Acetonitrile		
Gradient	Time (min)	Mobile phase A	Mobile phase B
	0-3	20	80
	3-7	50	50
	7-10	50	100
	10-13	0	100
Flow rate	1.5		
UV detection	220nm		
Injection volume	20 µl		
Column temperature	50°C		
Sample Temperature	4°C		
Run Time	13		
Dilution Solvent	Methanol		

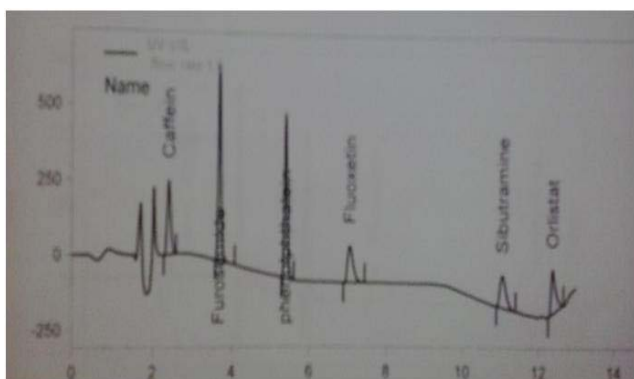


Figure 1: Chromatogram Chart (a)

RESULTS AND DISCUSSION

The chromatographic method is able to screen 6 weight loss drugs potentially present as adulterants in slimming dietary supplements or natural slimming formulations in less than 13 min. These drugs exhibit rather different physiochemical characteristics, so the mobile phase composition was changed through run depending on the substances' polarities and the method was able to detect

substances with distant polarities from caffeine to orlistat which to elute from column the plateau was brought to 100% acetonitrile. Figure 1 shows the separation of 6 selected weight-loss drugs using chromatographic parameters reported in Table 1.

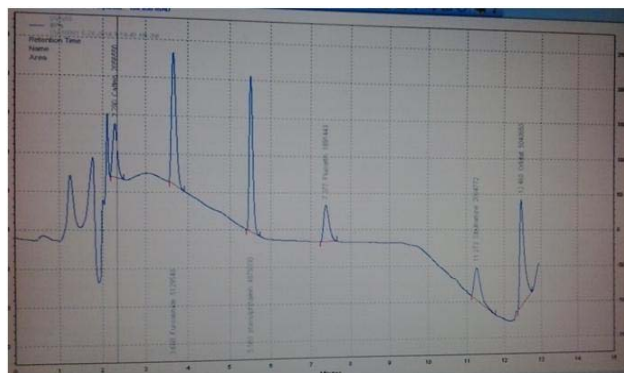


Figure 2: Chromatogram Chart (b)

Validation of the developed method

The proposed method was validated as per guidelines in ICH for its linearity, accuracy. Precision, specificity, selectivity and robustness.

Linearity, Limit of quantification and limit of detection

The linearity was tasted for the concentration range of 40, 45, 50, 55, 60 µg/ml and the calibration curve was constructed and evaluated by its correlation coefficient.

The correlation coefficient (r²) for all the calibration curves was consistently ≥ 0.995.

The equations of linear regression were performed using least-squares method.

The limit of quantification (LOQ) was the lowest concentration assayed where the signal/noise ratio was at least 10:1 The limit of detection (LOD) was defined as a signal /noise ratio of 3:1.

Table 2: Result of Linearity, LOQ, LOD

Parameter	Result		
	Caffeine	Furoseamide	Phenolphthalein
R ²	0.997	0.995	0.999
Equation	Y=643766x+5E+06	Y=1E+06x+9E+06	Y=1E+06x+8E+06
LOQ	0.75	0.25	0.1
LOD	2.25	0.75	0.3

Table 3: Result of Linearity, LOQ, LOD

Parameter	Result		
	Sibutramine	Fluoxetine	Orlistat
R ²	0.998	0.996	0.997
Equation	Y=541767x+4E+06	Y=426876x+3E+06	Y=650479x+4E+06
LOD	0.5	0.25	0.05
LOQ	1.5	0.75	0.15

Accuracy and Precision

The accuracy of the proposed method was tested by recovery experiments by adding known amounts of each materials (caffeine-furosemide-phenolphthalein-sibutramine-fluoxetine-orlistat) drug corresponding to 80,100 and 120% of the from the respective standard solution.

The accuracy was then calculated as the percentage of each six drugs recovered by this assay. The precision of the proposed method was assayed by replicate injections of six drugs mixture of three different concentrations (40, 50 and 60µg/ml), three times on three different days. The obtained intra-day and inter-day precision results are depicted in table 4.

Table 4: Recovery studies

Compound	Labeled	Amount added			Recovery %
		80 %	100 %	120 %	
Caffeine	50	40	50	60	100.95%
Furosemide	50	40	50	60	101.6%
Phenolphthalein	50	40	50	60	100.14%
Sibutramine	50	40	50	60	100.9%
Fluoxetine	50	40	50	60	101.58%
Orlistat	50	40	50	60	101.95%

Table 5: System suitability parameters

Parameter	Result					
	Caffeine	Furosemide	Phenol	Sibutramine	Fluoxetine	Orlistat
Retention time (min)	2.51	3.9	5.54	10.5	7.18	12.5
Theoretical plates	3507	15401	28048	21902	12374	48277
Tailing Factor	1.35	1.46	1.46	1.72	1.61	1.98

Specificity and Selectivity

The method specificity was assessed by comparing the chromatograms obtained from mixture of the drugs and the most commonly used excipients with those. At this study we have chosen national products, used as natural slimming capsules that it's scanning showed it doesn't contain any of substances that we have scanned for, so we used capsules' content of this product as excipient. This excipient was used to check the interference from Figure, it can be seen that the method was sufficiently specific to the analytes. The resolution factor for the drug peaks was founded to be more than 2 from the nearest resolving peak for all peaks and no interference were found in the retention of drugs. The system suitability parameters like tailing factor and number of theoretical plates were also calculated.

Robustness

The robustness was evaluated by deliberate variations of the method parameters. The factors selected to examine were flow rate (ml/min), PH of mobile phase and temperature °C.

One factor was changed at a me to estimate the effect. Flow rate variants 1.4and 1.6ml/min, the PH of buffer variants 4.8 ± 0.05 and 5.2 ± 0.05 .temperature variants: 48°C and 52°C.The retention time of the compound was evaluated, and the resolution had no significant changes when the parameters were changed precision data.

Table 6: Precision results

Compound	RSD%	
	Intra-day	Inter-day
Caffeine	0.86	1.35
Furosemide	1.75	1.56
Phenolphthalein	0.7	1.13
Sibutramine	1.24	1.53
Fluoxetine	1.03	1.42
Orlistat	1.69	2

CONCLUSION

A simple and accurate method was developed for the separation and simultaneous determination of adulterants drugs caffeine, furosemide, phenolphthalein, sibutramine, fluoxetine and orlistat. The proposed method has been validated by good linearity, precision, accuracy and robustness. The mobile phase was easy to prepare with little or no variation and was economical. The analysis time was found to be less than 13 min. The recovery from formulations was in good agreement and they suggested no interference in the estimation. Hence this method can be easily and conveniently used for detecting adulterates dietary supplements and herbal preparations used for slimming purposes with any of these six drugs.



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Source of Support: Nil, Conflict of Interest: None.

