SIMULTANEOUS DETERMINATION OF 34 WEIGHT-LOSS SUBSTANCES IN SLIMMING PREPARATIONS BY UHPLC WITH DIODE-ARRAY DETECTION

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INTRODUCTION

A huge quantity of illegal slimming products, such as tainted dietary supplements, moves on the market and may appear to be responsible of health troubles, including lethal cases. A general strategy to perform analysis on illegal products starts with the screening for a wide range of compounds. This paper deals with a methodology allowing the qualitative screening of 34 weight-loss compounds in slimming preparations such as medicines or dietary supplements. Quantification is performed using the standard addition approach. The Ultra-High Pressure Liquid Chromatographic system used a gradient of solvents (phosphate buffer 50 mM, pH 3.8 – acetonitrile), a trifunctional C18 column, fully endcapped and bonded to ethylene bridged hybrid substrate (Waters Acquity BEH C18 1.7 µm, 100 x 2.1 mm), and a diode array detector. This system allows identification based on retention time and UV spectra.

LIST OF THE 34 WEIGHT-LOSS SUBSTANCES

Anorectics
- Amfepramone (*2)
- Fenfluramine
- Phentermin (*2)
- Rimonabant
- Sibutramine (*3)

Stimulants
- Amphetamine
- Caffeine
- Ephedrine (*1)
- Metformine
- Synephrine
- Yohimbine

Antidepressants
- Fluoxetine
- Phenobarbital
- Penfluridol

Laxatives
- Phenolphtaleine

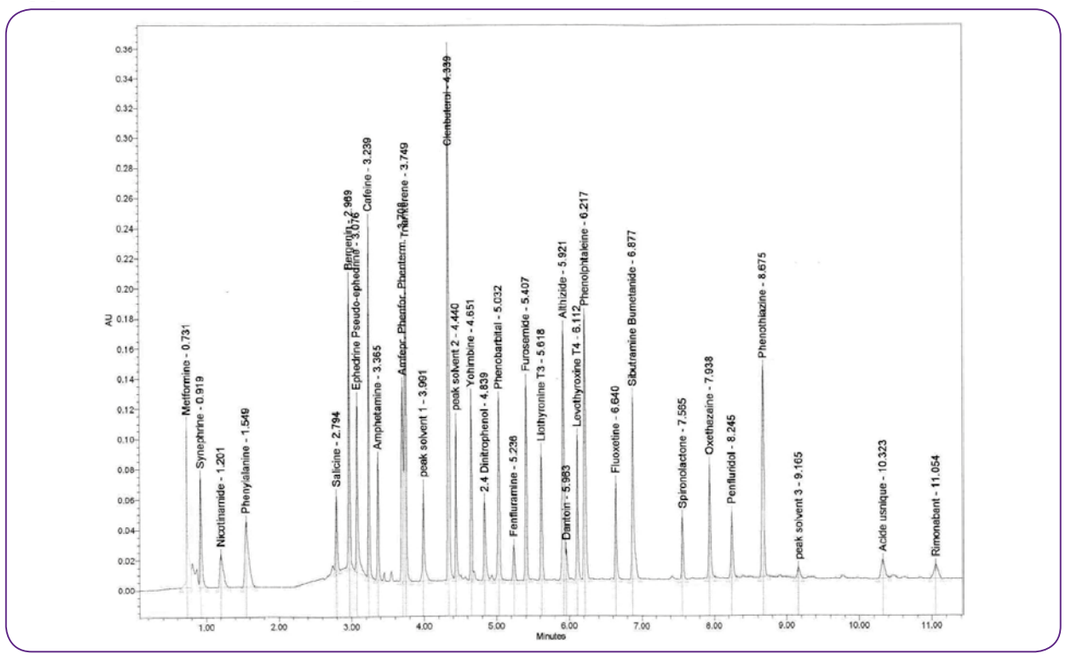
Diuretics
- Althiazide
- Bumetanide (*3)
- Furosemide
- Spironolactone
- Triamterene (*2)

Vitamins
- Nicotinamide

Others
- 2.4-Dinitrophenol
- Bergenin
- Clenbuterol
- Dantoin
- Levothyroxine (T4)
- Liothyronine (T3)
- Oxethazaine
- Phenformin (*2)
- Phenothiazin
- Phenylalanine
- Pseudoephedrine (*1)
- Salicine
- Usnic acid

(*1,2 and 3) : coeluted compounds

Application to 34 weight-loss substances



SAMPLE PREPARATION

For medicines and dietary supplements, one unit (tablet or capsule) was finely powdered and dispersed in 20 ml with a dilution solvent prepared by mixing 5 volumes of acetonitrile with 95 volumes of mobile phase A (phosphate buffer solution). The suspension was mechanically stirred during 15 minutes, sonicated 15 minutes more and then centrifuged during 15 minutes at 3500 r/min. The supernatant was filtered through a 0.45 m pore size GHP membrane filter (Pall-Gellman) discarding the first milliliter. Solutions were diluted according to (1:10; v/v) ratio in solvent mixture before analysis. For powder preparations, this protocol was adapted case by case.

ELEMENTS OF VALIDATION

Compound	λ max (nm)	Retention time (min)	Resolution (maxplot)	Symmetry (maxplot)	Repeatability %RSD (λmax)	Linearity Range (µg/ml, n=5)	r^2	LOQ (µg/ml)
Synephrine	222-273	0.92	7.5	1.8	1.5	0.1 – 10.7	0.999	0.1
Nicotinamide	214-261	1.20	6.4	1.2	0.4	0.5 – 50.0	1.000	0.1
Ephedrine	256	3.08	1.6	1.3	0.1	5.0 – 50.0	1.000	5.0
Caffeine	272	3.24	3.8	1.3	0.1	0.1 – 10.6	0.999	0.1
Amfepramone	252	3.71	9.4	1.3	0.4	0.1 – 21.0	0.999	0.1
Yohimbine	220-271	4.65	6.8	1.3	1.2	0.2 – 10.2	0.999	0.2
Fenfluramine	263	5.24	3.9	1.3	0.7	5.0 – 20.1	1.000	5.0
Phenolphthalein	229-275	6.22	3.4	1.2	0.5	1.0 - 50.0	1.000	0.8
Sibutramine	223	6.88	5.9	1.4	1.5	0.3 – 21.6	0.999	0.3
Rimonabant	232-282	11.05	1.5	1.0	0.5	1.0 - 50.0	0.999	0.8

Elements of validation have been carried out using standards in dilution solvent showing good selectivity (Rs> 1.5) between most of the 34 compounds, coelutions being overridden by differences on UV spectra.

CHROMATOGRAPHIC CONDITIONS

Column	Acquity BEH C18 1.7 µm, 100 x 2.1 mm		
Mobile phase A	Sodium dihydrogen phosphate 50 mM buffer (pH 3.8)		
Mobile phase B	Acetonitrile		
Gradient	Time (min)	%A	%B
	0	95	5
	1	95	5
	8	35	65
	13	35	65
	14	95	5
	15	95	5
Dwell volume	160 µl		
Flow rate	0.35 ml/min		
UV detection	Maximum absorbance (Maxplot)		
Injection volume	10 µL		
Column temperature	30°C		
Sample temperature	6°C		
Dilution solvent	Acetonitrile / mobile phase A (5/95) V/V		

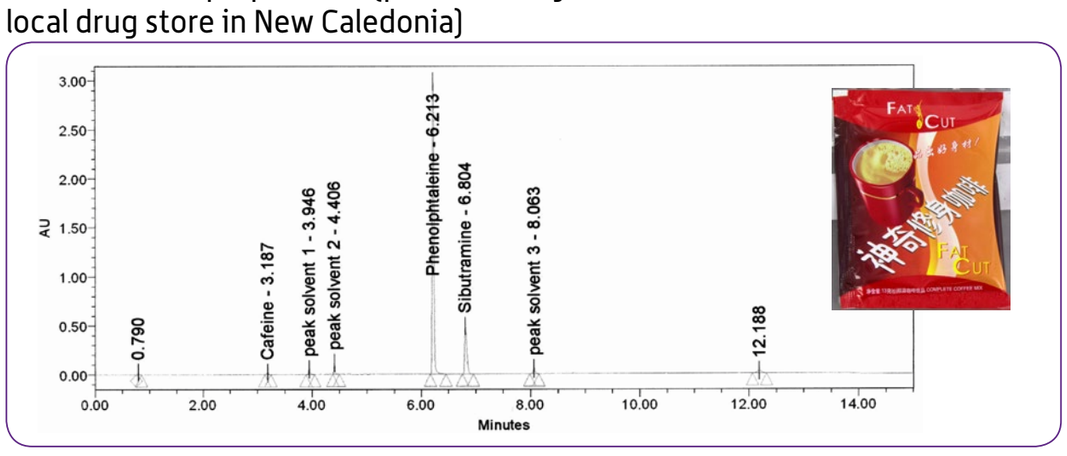
SCREENING METHODOLOGY

Step 1: suspicion of the presence of weight-loss substances
> RT and UV spectrum comparison (standard and screening sample solutions)

Step 2: confirmation step and assay
Analysis of a solution spiked with the suspected compound
Standard addition quantification

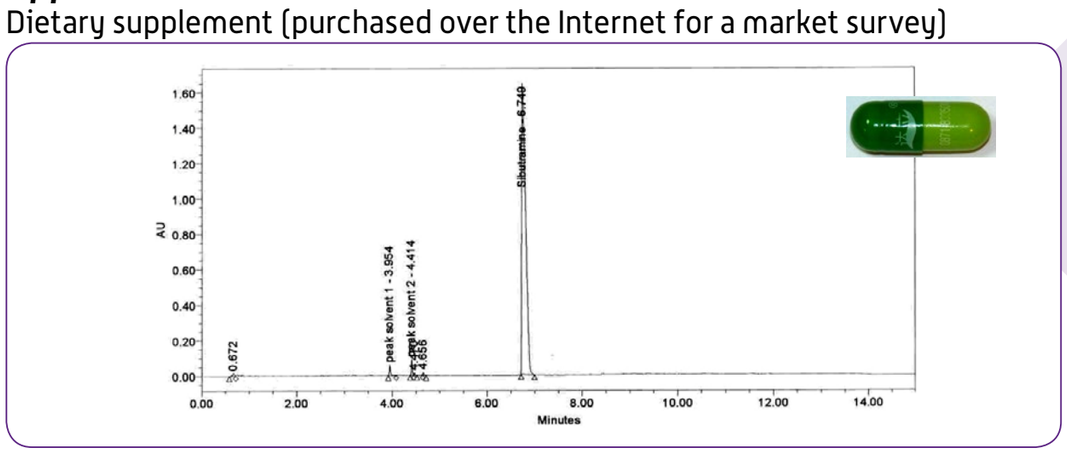
Application 1

Instant coffee preparation (purchased by French local health authorities from local drug store in New Caledonia)



Application 2

Dietary supplement (purchased over the Internet for a market survey)



CONCLUSION

The method enables the screening of 34 weight-loss compounds in complex matrices in less than 15 minutes. Elements of validations on standards in solvent have been obtained to ensure the reliability of the results. Using the screening methodology and the method of standard addition for quantification, numerous samples (including medicines, dietary supplements, small scale preparations, coffee powder) have been analysed in the laboratory leading to the determination of 7 weight-loss substances (caffeine, synephrine, yohimbine, sibutramine, phenolphthalein, rimonabant and nicotinamide) and also a sibutramine derivative (N,N-didesmethylsibutramine).

The UHPLC/DAD method described is simple, fast and selective for the determination of forbidden and harmful chemical compounds in slimming preparations.

REFERENCES

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