APPLICATION NOTE

Detection of sibutramine in slimming products by HPTLC and confirmation by HPTLC-MS

A-100.1

Keywords

Weight loss, adulteration, diet food, densitogram, mass detection

Introduction

Diet foods, such as slimming coffee or dietary food supplements, are widely marketed and numerous preparations are available to consumers through several distribution channels. They are particularly easy to obtain via the Internet. The fraudulent addition of sibutramine has recently been detected in many tainted natural slimming pills and is of serious health concern.

Scope

This method is suitable for the identification of sibutramine in finished products. The TLC-MS interface 2 was used to extract target zones from the HPTLC plates to confirm the presence of sibutramine by MS, especially for samples with a low concentration of the adulterant.

Required or recommended devices

Automatic TLC Sampler 4 or Linomat 5, Automatic Developing Chamber ADC 2, TLC Visualizer, TLC Scanner 4 or 3, visionCATS software, TLC-MS Interface 2, Waters ACQUITY QDa Detector (Performance), Empower® or MassLynx® software.

Sample

200 mg of the entire capsule is dissolved in 10 mL of methanol. Samples are homogenized for 30 s by vortex mixing and extracted in an ultrasonic bath for 10 min at room temperature. After centrifugation at 2750 RCF for 10 min at 25°C, the supernatant is collected and used as test solution.

Standards

Sibutramine reference material was purchased by Fluorochem (Derbyshire, UK) and prepared at a concentration of 0.785 mg/mL in methanol.

Chromatography

Stationary phase:	HPTLC Si 60 F254, 20 x 10 cm (Merck)
Sample application:	Bandwise application, 15 tracks, band length 8 mm, track distance 11.4 mm, distance from left edge 20 mm, distance from lower edge 8 mm, application volume of 2 µL of standard, and 5 µL of each sample.
Developing solvent	Toluene, methanol 9:1 (v/v) (according to [1])
Development	In the ADC 2 with chamber saturation (with filter paper) 20 min and after

	conditioning at 33% relative humidity for 10 min using a saturated solution of magnesium chloride
Developing distance	70 mm (from the lower edge)
Plate drying	Drying 5 min in the ADC 2
Documentation	With the TLC Visualizer under white light (after derivatization)
Densitometry	Densitometric analyses are performed at 225 nm in absorption mode, at a scanning speed of 20 mm/s using a slit of 5.0 × 0.3 mm.
MS confirmation	Localizing the sibutramine zone on the HPTLC plate: Marking zones to be eluted by using TLC Visualizer under UV 254 nm (enhanced image), or based on RF values of reference substance obtained by densitometry. Target zones are directly eluted using the TLC-MS Interface 2 with oval elution head into the ACQUITY QDa Detector at a flow rate of 0.5 mL/min with acetonitrile (with 0.1% formic acid). For a full scan spectrum it is recommended to first elute a blank, which can be subtracted from the spectra of the target zones. There is no need for Single Ion Recording (SIR).
MS parameter	The ACQUITY QDa Detector is operated in ESI+ mode using default settings. The ESI capillary is set to 0.8 kV, cone voltage to 15 V, and desolvation temperature at 600 °C. A full scan mass spectrum between m/z 50 and 650 is acquired at a sampling rate of 10.0 points/sec (continuum). Data processing and evaluation of mass spectra are performed with Empower. For routine use in quality control Single Ion Recording (SIR) can be performed.

Results

System Suitability Test (SST) by densitometry at 225 nm:
Sibutramine zone at R_F ~ 0.46

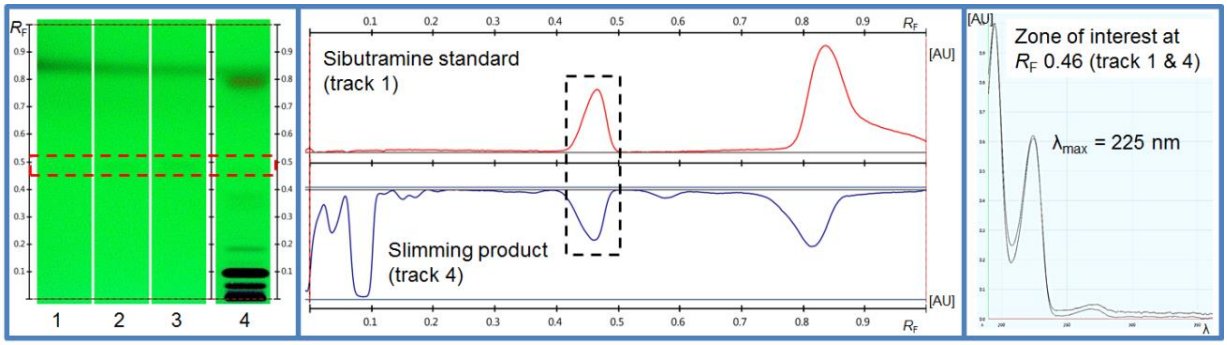

Fig. 1 Left: Plate under UV at 254 nm (contrast 4.0; exposure: 0.320 s), tracks 1-3: standard (2 µL, 4 µL, 6 µL), track 4: slimming product (5 µL); middle: scan at 225 nm, right: UV spectra of interested zones

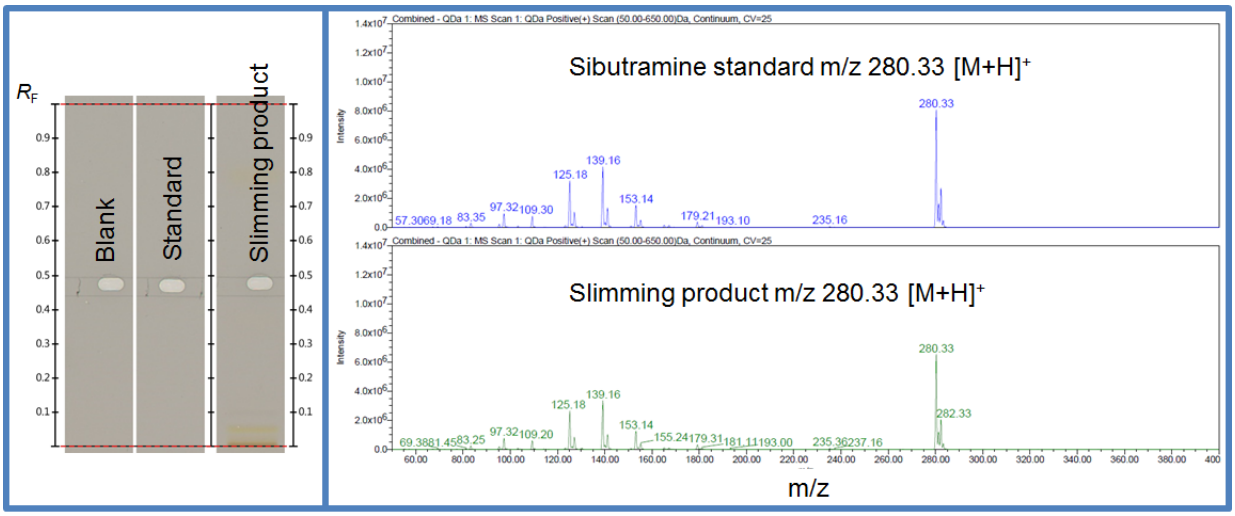

Fig. 2 HPTLC-MS spectra of sibutramine (standard), and sibutramine (slimming product), displayed range m/z 50 to 400

Literature

[1] Mathon C., Ankli A., Reich E., Bieri S., Christen P.: Screening and determination of sibutramine in adulterated herbal slimming supplements by HPTLC-UV densitometry. Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess., 2014, 15-20.