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dtoLABS Analytical Excellence Center
Agilent Technologies Authorized Partner Laboratory

Analytical strategy for the detection of sibutramine in dietary supplement by 6550 iFunnel Q-TOF LC-MS

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Introduction & aim

Adulteration of botanical food supplements with undeclared synthetic drugs is becoming a widespread and mostly uncontrolled problem in many countries [1-5]. Among them, slimming functional food are commercially readily available to a vast unaware population. At the moment, there are no established analytical protocols for the systematic detection of synthetic adulterants in these products but a large body of literature is converging to the target screening approach, either by liquid chromatography or gas chromatography [6, 7]. However, this approach may not be suitable due to the sheer number of chemicals. For this reason, high-resolution high-accuracy mass spectrometry (HRMS), enabling accurate-mass determination of ionic species (and metabolites), offers the potential to overcome the limitations of multi-target screening.

Aim

The concept of HRMS is not novel at all and in recent years its use has become more widespread due to technological improvements. The present work shows a simple but effective approach to detect sibutramine and caffeine in allegedly 'natural' herbal extracts by Q-TOF LC/MS technology.

Materials & methods

LC Parameters	
Instrument	Agilent 1290 LC System
Column	Agilent ZORBAX Eclipse Plus C18, RRHT 2.1 mm x 150 mm, 1.8 µm
Mobile Phases	A: water +0.01% formic acid B: methanol +0.01% formic acid
Flow rate	0.2 mL/min
Temperature	40°C
Injection Volume	5 µL
Gradient:	Time (min) % B
	0-1 5
	1-12 95
	12-15 95
	15.1 5
Post Run Time	3 minutes

MS Parameters	
Instrument	Agilent 6550 iFunnel Q-TOF LC/MS
Ionization Mode	ESI +
Drying Gas Temperature	125°C
Drying Gas Flow Rate	20 L/min
Nebulizer Gas Pressure	40 psi
Sheath Gas Temperature	325°C
Sheath Gas Flow Rate	12 L/min
Vcap Voltage	3500 V
Nozzle Voltage	300 V
MS scan centroid + profile	40-1000 m/z, rate 1 spectra/sec, 5993 transient/spectrum
MS ALL ion	40-1000 m/z, rate 1 spectra/sec, 5993 transient/spectrum Exp1 CE 0V Exp2 CE 10V Exp3 CE 20V Exp4 CE 40V
Target MS/MS	Sibutramine: m/z 280.1826, RT 10.4 ± 1 min CE 10V CE 20V CE 40V MS acquisition rate 1 spectrum/sec, 8154 transient/spectrum MS/MS acquisition rate 3 spectra/sec, 2663 transient/spectrum

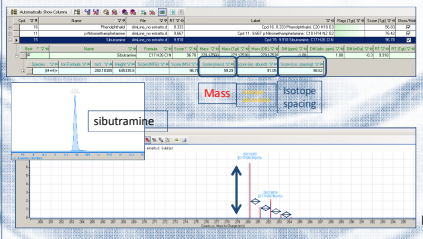
SAMPLE PREPARATION

Figure 1. Samples are reported in this picture, all of them were commercially freely sold.

For screening, powders (1&3)and liquid samples (2, soft-gel capsules) were weighted to 400 mg and suspended in 16 ml of water under agitation. After centrifugation, 50 µl of sample was diluted in 150 µl of water and directly injected. A second aliquot of 1 ml of each sample was LLE with ToxiTube A devices. The surnatant was dried and then reconstituted in 200 µl of water. For quantification, samples were diluted 1/10, 1/100 and 1/1000 in water. External standard calibration curves were used to quantified the confirmed analytes.

Results

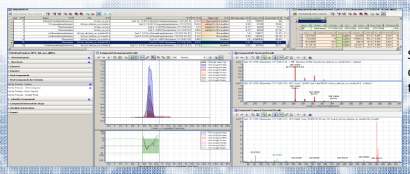
Dissolved and diluted samples were firstly acquired in SCAN mode according the described analytical parameters. The data mining algorithm Find by formula and DB research with fixed Δppm 10 and [M-H+] adduct were used to screen samples for the compounds present in PCDL. Blank injections were used to eliminate false positive results from the system. Results showing a score > 80% were considered consistent and further investigated.



The score of a compound or spectrum is based upon the score for each of the identification techniques applied to it. The contribution to overall score is calculated by the SW from the Mass Match, isotope abundance score, spacing match and retention time score (Fig. 2). Following this way, sibutramine was found as a preliminary identification result in sample 1 and sample 2 with Mass and Isotope scores greater than 91% and Δppm = 1.08 (Fig. 2). Caffeine and other compounds were also preliminary identified.

Figure 2.

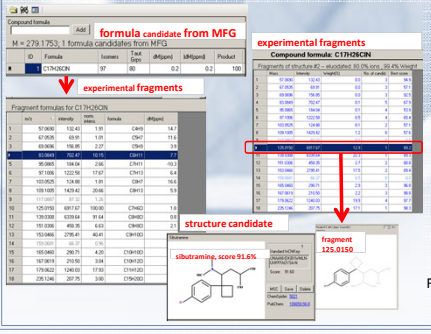
Samples were then analyzed in ALL ION acquisition mode in order to consider the accurate mass fragments and consider the coelution scores values contribution to the preliminary analytes identification. Sibutramine and caffeine were still evaluated as possible candidates also for the presence of significative fragments in ALL ION acquisition mode (Fig. 3).



Seven sibutramine fragments were confirmed by good coelution scores values (> 70%) and a Δppm= 2.5 between target and measured mass was achieved.

Figure 3.

Due to the risks posed to the health by undeclared sibutramine presence, further efforts were taken and Target MS/MS runs were executed in order to acquire spectral information ; "find by target MS/MS" data mining algorithm was used with averaged background subtraction to the acquired data. Then MFG (Molecular Formula Generator) was applied to the unknown compound, in order to generate a panel of possible formulae brute from the acquired spectra, resulting in the formula C17H26ClN with overall score 97.17% and Δppm 0.39.
Theoretical and experimental fragment correlation was obtained with a supplemental tool, MSC (Molecular Structure Correlator). MSC tries to explain each observed fragment ion into the proposed structure using a "systematic bond-breaking" approach as described by Hill and Mortishire-Smith [8]. The input for MSC is an accurate mass MS/MS fragment spectrum, a molecular formula for the compound of interest, and one or multiple candidate molecular structures. The MSC then uses the selected formula, retrieves one or multiple possible structures from a .mol file, an .sdf file, a MassHunter compound database (PCD, PCDL) or ChemSpider (web) and scores how well each candidate structure correlates with the MS/MS spectrum. The overall correlation score gets calculated from individual scores for each fragment ion signal. For each fragment ion one or multiple substructure candidates may be suggested and a "penalty" assigned based on how many and that bonds need to be broken to generate that substructure. Other factors impacting the overall correlation score are the mass accuracy of the observed fragment ions and the overall percentage of fragment ion intensity that can be plausibly explained with substructures.



MSC results obtained for sibutramine are depicted in fig. 4. Theoretical structures retrieved for fragments comparison were from .mol file (for sibutramine), toxicological PCDL and ChemSpider. In all cases, sibutramine has been recognized as best candidate.

Figure 4.

Conclusions

Compound identification was obtained by matching accurate mass, retention time (if available) and CID fragmentation patterns data. In this way, caffeine and sibutramine were confirmed and quantified. The limits of detection (LOD), defined as S/N= 5 were 0.5 and 2 ng/ml for sibutramine and caffeine respectively; limits of quantification (LOQ), defined as S/N= 10 were 1 ng/ml for sibutramine and 4 ng/ml for caffeine.
Sibutramine was found in two out of the three analyzed samples at a concentration of 0.6 and 1.04 µg/mg. The caffeine content varied from 5 to 100 mg per sample/pack.

Final results

Compound	Formula	RT	Sample #	ALL ION Confirmation	Standard Confirmation	Results
Caffeine	C8H10N4O2	6.5	1	yes	yes	0.1 %
Caffeine	C8H10N4O2	6.5	3	yes	yes	2.0 %
Cathine or 4-hydroxyamphetamine	C9H13NO	6.3	2	no	no	
Ephedrine	C10H15NO	5.5	3	no	no	
MDA	C10H13NO2	4.3	2	no	no	
Sibutramine	C17H26ClN	10.5	1	yes	yes	15 µg/mg
Sibutramine	C17H26ClN	10.5	2	yes	yes	26 µg/mg
Theophylline	C7H8N4O2	5.7	1	yes	Not tested	
Trimethoxyamphetamine	C13H21NO3	3.4	2	no	no	

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