

# Use of X500R QTOF for Monitoring Unexpected Additives in Nutritional Supplements

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## Introduction

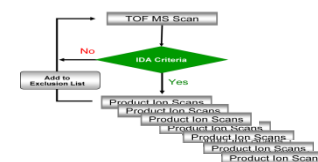
Nutritional supplements can supplement necessary nutrients and are believed to support recovery from illness. Generally, these products promote a particular effect or claimed function<sup>[1]</sup>; thus, in typical use, people often incorrectly believe they have a definite treatment efficacy. They are often linked to the alleviation of certain illnesses. In order to maximize these functions, manufacturers may add related drugs in order to increase their efficacy without including them as a listed ingredient. According to reports and discoveries from actual monitoring cases, unexpected additives to nutritional supplements are generally selected because they relate to the health product effects or address the additive side effects or functions; the additive usually takes the form of one or more drug additives, drug derivatives, etc.<sup>[5]</sup> Because these additives are generally high-dose, drug interactions can be unclear. Thus, a great potential hazard exists for human health<sup>[2-4]</sup>; the China Food and Drug Administration (CFDA) "Health product potential illegal additives list" clearly stipulates monitoring processes for additives in 6 different types of nutritional supplements: those with weight loss, blood sugar reduction, blood pressure reduction, anti-fatigue, sleep improvement, and immune strengthening functions. The purpose is to protect consumers' health.

SCIEX's X500R QTOF high resolution mass spectrometry system can be used for rapid monitoring of additives in nutritional supplements; after sample injection, a first order mass accuracy number and second order fragmentation spectrum are simultaneously obtained. Currently, over 50 additives can quickly be qualitatively confirmed in this way. Matrix interference in complex matrices can be overcome for specific screening of additives; preprocessing is even simpler and more convenient. The new SCIEX OS software fully integrates instrument control, data collection, data handling, and other processes. The workflow is more intuitive and smarter; this method provides an efficient means for rapid, high-throughput monitoring of nutritional supplements for additives.

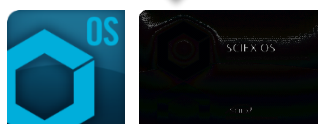
## Experimental Process

1. Collect samples of 6 types of nutritional supplements currently on the market - those with weight loss, blood sugar reduction, blood pressure reduction, anti-fatigue, sleep improvement, and immune strengthening functions. Perform simple preprocessing.
2. Use TOF MS-IDA MS/MS mode for data collection; after sample injection, obtain first order ion and second order ion fragmentation spectrograms.
3. The mass accuracy number, isotope distribution, retention time, and standard library alignment are used for positive verification of samples and checking the accuracy of sample monitoring results.
4. Monitoring reports systematically summarize sample screening results; the report content can be tailored to specific requirements.

## X500R high-resolution mass spectrometry screening workflow:



1. Both TOF-MS-IDA-MS/MS And TOF-MS/MS data gathered in the same injection



2. SCIEX OS is the integral software used to perform this analysis



3. Screening results and report generation

## Preprocessing Method

1. Use tablets ground into a powder, granules from inside capsules, or liquid samples; weigh accurately a 1.0g sample, and place in a 10mL centrifuge tube;
2. Add 5mL acetonitrile and agitate 2 min;
3. Vortex 2 min;
4. Centrifuge at 4°C at 10000 Rpm for 15min;
5. Dissolve the supernatant 1-fold;
6. Pass through a 0.22µm filter and directly inject sample;

## Liquid Phase Conditions

Chromatographic Column: Phenomenex Kinetex C18,  
2.1\*100mm, 2.6µm;

## Elution gradient

Time (min)	A%	B%
0	95	5
5.0	55	45
15.0	20	80
20.0	5	95
25.0	5	95
25.1	95	5
30	95	5

**Positive ion mode:** A: 0.1% Formic acid Water; B: 0.1% Formic acid Acetonitrile;

**Negative ion mode:** A: Water; B: Acetonitrile;

**Flow rate:** 0.3mL/min;

**Column temperature:** 40°C;

**Amount inserted:** 10 µL;

## Mass Spectrometry Method

**Scanning method:** TOF MS-IDA MS/MS

**Ion source:** ESI source

**Scanning range:** m/z 50-2000

**CUR gas:** 30 PSI

**Collision gas CAD:** 7

**IS voltage:** 5500V/-4500V

**Source temperature:** 600°C

**Atomizing gas GAS1:** 55 PSI

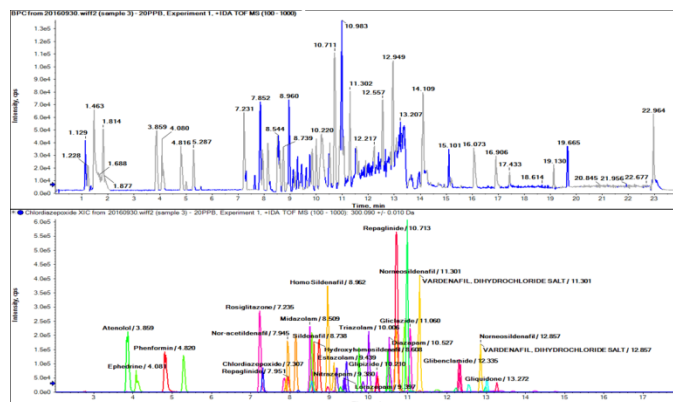
**Auxiliary gas GAS2:** 70 PSI

**DP voltage:** ± 60V

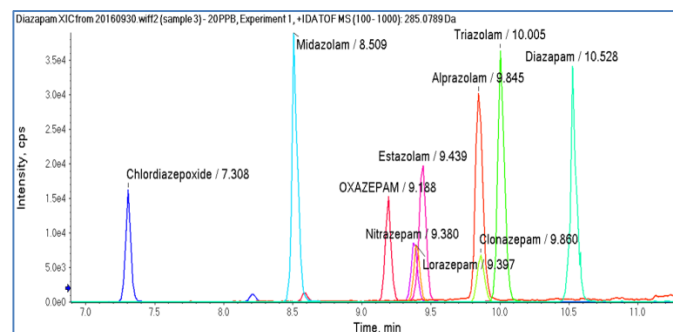
**Collision energy:** 35 ± 15V

## Unexpected Additive Screening Method

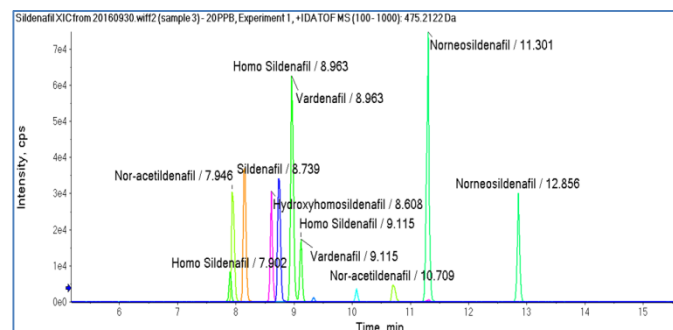
Injection of a single sample simultaneously monitors for over 50 unexpected additives:



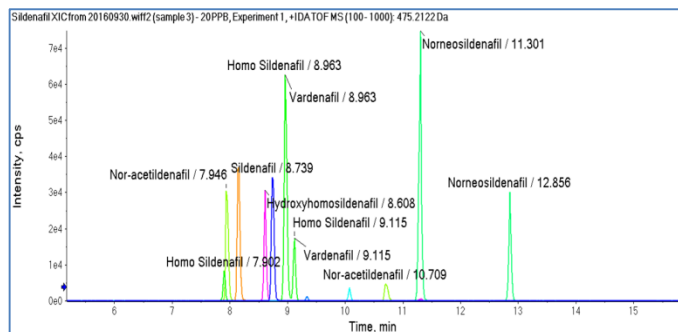
1. 10 sedative-hypnotic mixtures (20ppb), ion extraction flow diagram (XIC) appears below:



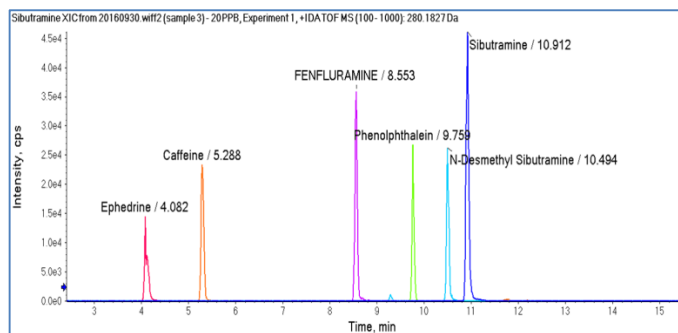
2. 7 blood glucose-lowering drugs (concentration 20ppb); ion extraction flow diagram (XIC) appears below:



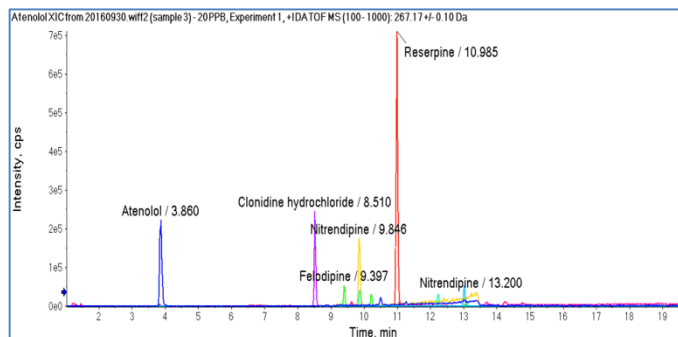
3. 8 impotence drug mixtures (20ppb), ion extraction flow diagram (XIC) appears below;



4. 6 weight loss drug mixtures (20ppb), ion extraction flow diagram (XIC) appears below;



5. 5 blood pressure-lowering drug mixtures (20ppb), ion extraction flow diagram (XIC) appears below;



## Sample Information

Following the CFDA's "Health product potential illegal additives list" 6 different nutritional supplements were randomly selected, including those for weight loss, blood sugar reduction, blood

pressure reduction, anti-fatigue, sleep improvement, and immune strengthening. Samples came from 19 different brands;

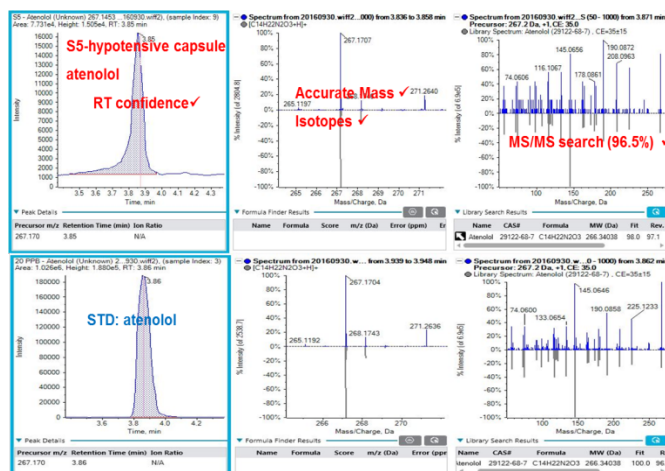
Sample No	sample type	name
Sample 1	sleeping	epiphysis pacify
Sample 2	hypoglycemic action	glycolipids safe
Sample 3	hypoglycemic action	hypoglycemic extract
Sample 4	anti-hangover	prime power
Sample 5	hypotensive	hypotensive capsule
Sample 6	sleeping	pacify syrup
Sample 7	hypoglycemic action	hypoglycemic TCM
Sample 8	slimming	slimming capsule
Sample 9	hypotensive	Hypotensive pill
Sample 19	.....	.....

## Experimental Results

### Blood Pressure-Lowering Drugs

#### 1. Sample no. 5 - atenolol positive

Sample no. 5 is a blood pressure-lowering capsule; it claims to have a rapid effect and prolonged use can control blood pressure.

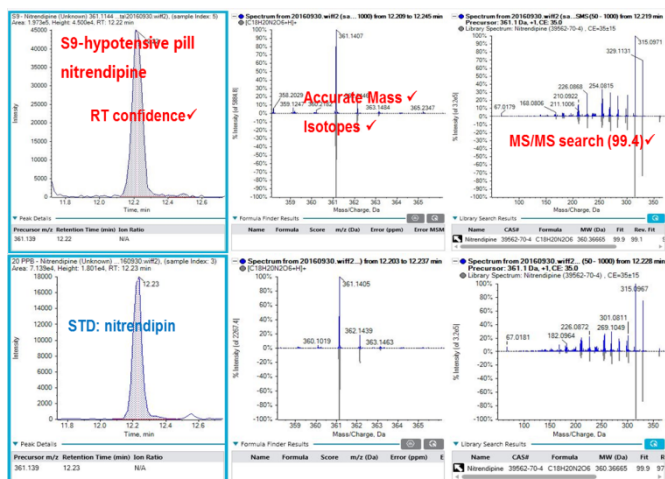


Screening with the X500R QTOF system showed Sample no. 5 contains large amounts of the additive atenolol. Prolonged use of high-dose atenolol can lead to serious side effects including decreased vision, breathing difficulties, weakness, depression, unexplained rash and ankle swelling and other symptoms.



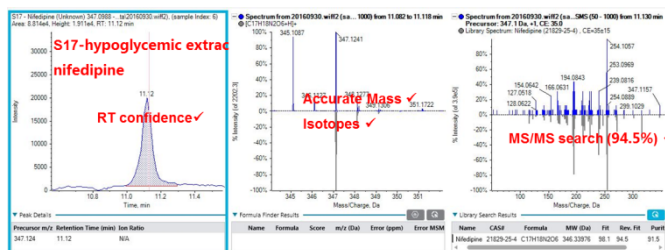
## 2. Sample no. 9 - nitrendipine positive

Sample no. 9 is from a brand of blood pressure-lowering tablet; screening shows a definite quantity of nitrendipine. The product claims to contain pure and natural extracts with no side effects, but prolonged oral nitrendipine can cause diseases like allergic hepatitis, rash, and even exfoliative dermatitis.



## 3. Sample no. 17 - nifedipine positive

Sample no. 17 is from a brand of blood pressure-lowering Chinese medicine; screening shows a nifedipine additive. It claims to lower blood pressure with Chinese medicine, falsely advertising an anti-hypertensive effect.

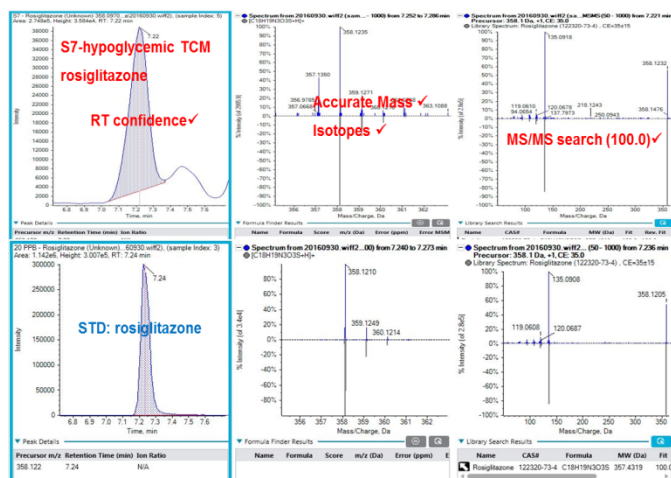
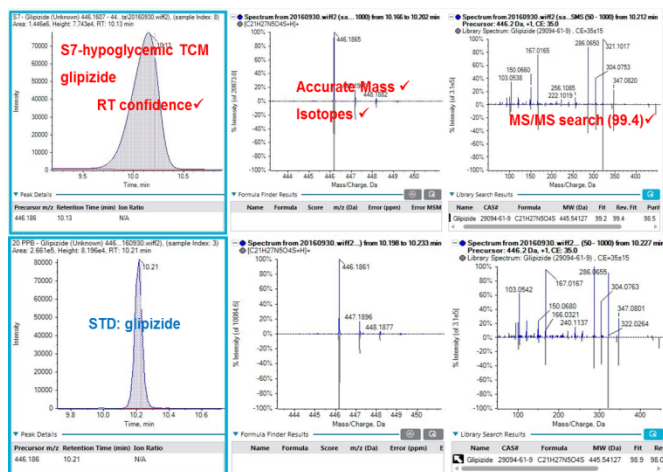
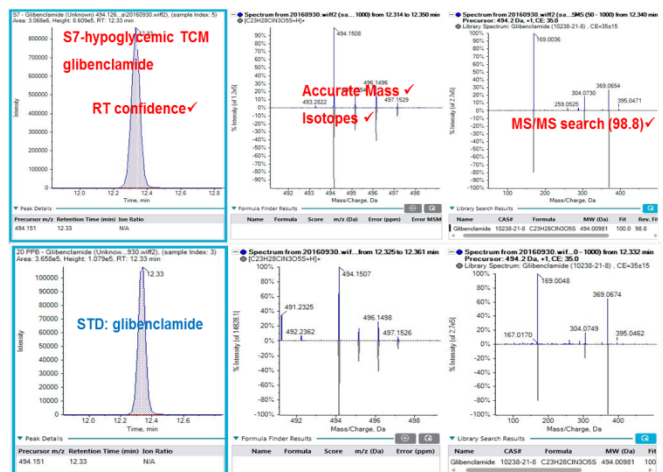


## Glucose-Lowering Drugs

### 1. Sample no. 7 - glibenclamide, glipizide, rosiglitazone positive

Sample no. 7 is a brand of glucose- and lipid-lowering capsule; test results show sample no. 7 contains the 3 glucose-lowering drugs glibenclamide, glipizide, and rosiglitazone as additives. Improper use of sulfonylureas such as glibenclamide and glipizide can cause hypoglycemia; patients can rarely develop rash, erythema multiforme, edema, and liver and kidney damage. Thiazolidinediones like rosiglitazone can cause slight

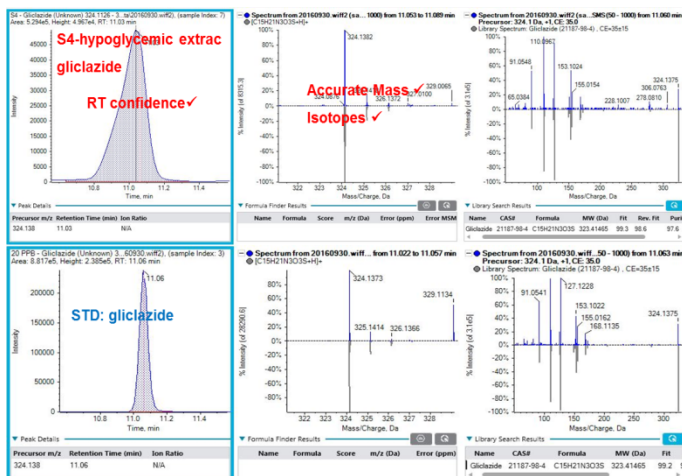
hypersensitivity and mild headache when used incorrectly or at improper doses.



### 2. Sample no. 4 - Glucalide positive

Sample no. 4 is a brand of plant extract; it is mainly used to stabilize blood sugar. Screening results show an addition of glucalide, which produces a definitive glucose-lowering effect.



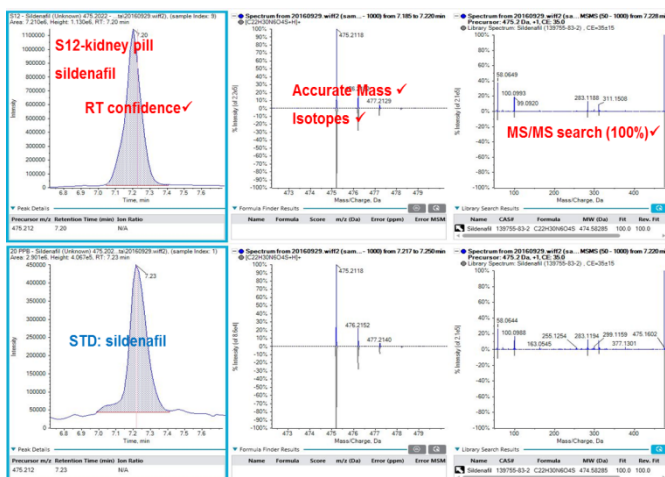


Glucose-lowering drugs are low-cost; they are common “functional components” added to nutritional supplements. These chemical drugs are often used to treat diabetes, as they have a clear hypoglycemic effect. However, their side effects are also quite evident; prolonged use can lead to hypoglycemia and kidney damage, even leading to death.

## Anti-Fatigue/Impotence

### 1. Sample no. 12 - sildenafil positive

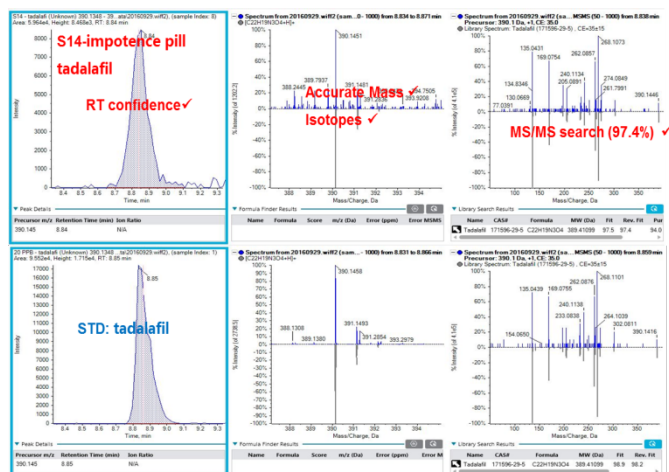
Sample no. 12 is a kidney health product for the elderly; its description states it is pure Chinese medicine and contains several flavors of medicine. Screening shows an addition of large quantities of sildenafil in order to achieve its claimed kidney effects.



### 2. Sample no. 14 - tadalafil positive

Sample no. 14 is a brand of impotence health product. Impotence products are the most frequently found to contain additives. In order to accelerate the speed of onset, additives are

generally used in large quantities; screening results showed sample no. 14 contained tadalafil.



When not used under the guidance of a specialized physician, prolonged use of nutritional supplements containing “impotence” additives can severely harm the body. Side effects can include dizziness, fainting, and even hearing loss.

Screening results appear in the table:

1. The problem of additives in nutritional supplements is widespread; additives appear in many samples;
2. Blood sugar- and pressure-reducing products contain many different additives; they generally take the form of multiple drugs, and use of Chinese medicine is especially serious.
3. Anti-fatigue and impotence health care products generally contain large amounts of additives;

Sample No	sample name	positvie results
Sample 1	epiphysis pacify	-----
Sample 2	glycolipids safe	-----
Sample 3	hypoglycemic action	-----
Sample 4	hypoglycemic extrac	gliclazide
Sample 5	hypotensive capsule	atenolol
Sample 6	pacify syrup	-----
Sample 7	hypoglycemic TCM	glipizide, rosiglitazone, glibenclamide
Sample 8	slimming capsule	-----
Sample 9	hypotensive pill	nitrendipine
Sample 12	kidney pill	sildenafil
Sample 14	impotence pill	tadalafil
Sample 17	hypoglycemic extrac	nifedipine

## Summary

This study randomly selected 19 nutritional supplements commonly found on the market; these covered 7 glucose- and blood pressure-lowering products, 5 anti-fatigue, anti-impotence products, 4 sleep aids, and 3 weight loss products. Screening results showed that blood pressure-lowering and glucose-lowering products most commonly contained additives, especially those products advertised to use Chinese medicine extracts to lower blood sugar. Representative samples of blood pressure-lowering capsules showed a high rate of positive results. The main additives were atenolol, nitrendipine, nifedipine, glibenclamide, glipizide, rosiglitazone, gliclazide and other inexpensive and readily available glucose- and blood pressure-lowering drugs, impotence, anti-fatigue/immune system-enhancing additives were generally sildenafil or tadalafil. Additives take the form of one or many drugs; some additives are present in amounts several times therapeutic doses. Thus, they can be quite hazardous to consumer health.

The SCIEX X500R QTOF high resolution mass spectrometry system was used for rapid monitoring of 50 different additives in 6 types of nutritional supplements. Its high sensitivity detected small concentrations of additives, its rapid scanning and effective overcoming of complex matrix interference ensure that after sample injection, a first order mass accuracy number (TOF-MS) and second order fragmentation spectrum (TOF-MS/MS) are simultaneously obtained. Combined with the high-quality additive library, accurate qualitative screening for additives in complex matrices can be performed.

Health product additive screening methods using the X500R QTOF system are reliable, simple, and rapid. The system provides an efficient approach to additive screening of nutritional supplements, and it ensures health and safety product quality; it is critical in the fight against the use of potentially harmful additives.

## References

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