Total Solution of QTRAP® Mass Spectrometry on Screening of Additives in 68 Common Chinese Health Products

Total solution of QTRAP on drug screening of health products

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Introduction

With the increase in living standards, so has the health products market. With fierce market competition, in an effort to provide quick results to consumers, some manufacturers have added drugs to health products in order to accelerate results. For instance, additives like sibutramine and phenolphthalein are found in many weight loss products. Adulterated health products may provide consumers with quick results, but they may also encounter side effects such as drug dependence, liver damage, and tachycardia. The China National Food Safety Supervision and Sampling Implementation Guidelines (2017 version) have established monitoring parameters for health products. This guide covers monitoring of 68 drugs in 6 different categories of health products.

In traditional mass spectrometry, MRM mode often produces matrix effects in practice due to matrix complexity. This leads to retention time and ion ratio discrepancies and causes “false peaks” and “false positives” that interfere with determinations. To overcome the challenges of MRM mode in traditional mass spectrometry, the SCIEX complex mass spectrometry QTRAP system uses a uniquely integrated MRM-IDA-EPI scanning mode. This provides MRM spectral peaks and enhanced secondary fragments with just one sample injection. MRM ion channel chromatographic peak quantification and EPI different energy complex secondary spectra form a “fingerprint” spectrum. The library is searched and verified, effectively overcoming the traditional mass spectrometry challenges and increasing the accuracy of testing and analysis work.

The following method was developed on the QTRAP platform to detect the 68 health products in the 2017 National Food Safety Supervision and Sampling Implementation Guidelines. It also includes a secondary library to help users search, identify, and enhance the efficiency of monitoring and analysis. This monitoring protocol includes these advantages:

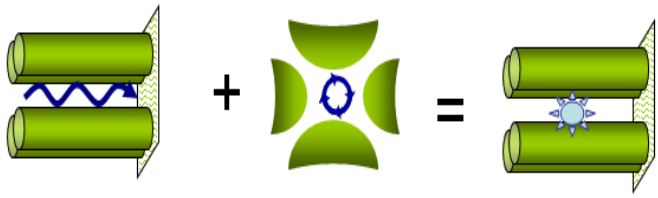
1. Covering all drug types, this method includes all drug additives to health products that must be detected per the 2017 National Food Safety Supervision and Sampling Implementation Guidelines.
2. With one sample injection and simultaneous positive and negative mode scanning, it is quick and easy.
3. This method includes sample preprocessing, MRM ion pair data, instrumentation methods, and secondary search libraries. The QTRAP comprehensive solution includes the advantage of simultaneous quantitative and qualitative validation with a single sample injection.
4. The QTRAP comprehensive solution satisfies multiple user needs; in use, it improves work efficiency and saves time.
5. Secondary search databases have high, medium, low, and combined energy fragment spectra. They contain a large amount of fragment information and effectively exclude false positives.

Experimental process

1. Integrated mass spectrometry with QTRAP involves one sample injection for simultaneous quantification and qualification. When the “fingerprint” of an “illegal additive” is detected, one can also obtain information on the content, as well as quantitative and qualitative validation data. This provides a novel workflow for analysis work.

Complex mass spectrometry with QTRAP® involves one sample injection for simultaneous quantification and qualification.

QTRAP series



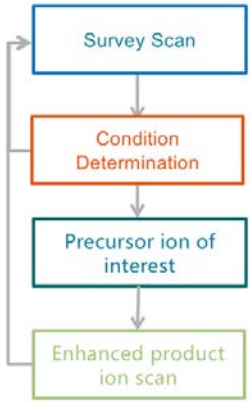
Triple Quadrupole
Satisfies the international legal requirement for Two pair MRM and MRM Ratio

Ion Trap
Can store ions, increase ion amounts
The enhanced spectrum satisfies the need for validation

QTRAP
Simultaneous qualification and quantification
Exclude false positives

2. QTRAP's unique MRM-IDA-EPI workflow is the solution required to identify drugs.

MRM triggered IDA principle



Procedure 1: Survey scan

Procedure 2: System automated determination: Intensity of survey scan signals exceeds expected values ("apparent chromatographic peaks")

Procedure 3: When Procedure 2 conditions are met, the system automatically and rapidly (<1 ms) switches to linear ion trap mode and performs Enhanced Product Ion (EPI) scanning to create high quality parent ion MS^2 spectra from survey scans. Return to Procedure 1.

3. Using library search, with positive and reverse matching, determine the overall degree of matching. Efficiently eliminate false positive results.

Library search flowchart

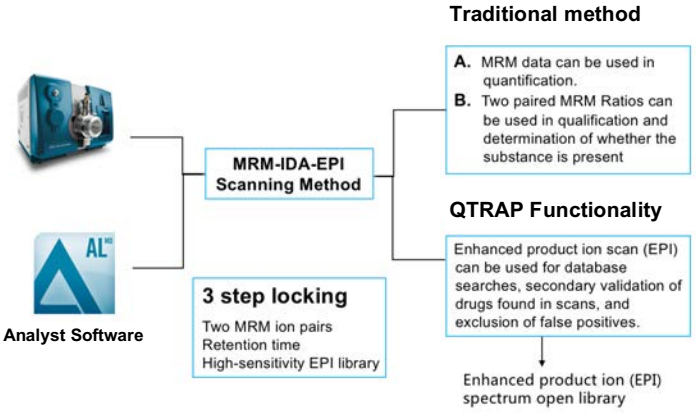
The ion pair of the substance to be measured is extracted from the MRM general spectrum to determine if the sample contains the substance.

If peaks occur in this substance, double-click the EPI spectrum of the corresponding retention time to obtain an enhanced secondary spectrum (EPI).

Enhanced (EPI) secondary spectra, right-click library search, positive sample validation, exclusion of false positives

Complex mass spectrometer QTRAP screening workflow

QTRAP screening workflow--- Analyst Software + database



Liquid phase conditions

Chromatographic Column:

Phenomenex Kinetex C18,2.6u,50×2.1mm

Mobile phase:

A: Acetonitrile

B: 5 mmol/L aqueous ammonium acetate solution

Gradient elution was performed as shown below:

Time（min）	A%	B%
0	90	10
10.0	10	90
12.0	10	90
12.1	90	10
15.0	90	10

Flow rate: 250 μL/min； Column temperature: 40℃；

Amount injected: 10 μL

Mass spectrometry method

SCIEX QTRAP 4500 triple quadrupole complex ion trap mass spectrometer

Scanning method: MRM full scanning mode

Positive, negative scheduled MRM mode simultaneous scan

Ion source: Turbo V™ ESI source

Mass spectrometry parameter establishment

ESI ion source parameters:

Air curtain gas CUR: 30psi; Collision gas CAD: High

IS voltage: 5500V/-4500V

Ion source temperature: 550°C

Atomizing gas GAS1: 55psi

Auxiliary gas GAS2: 55psi

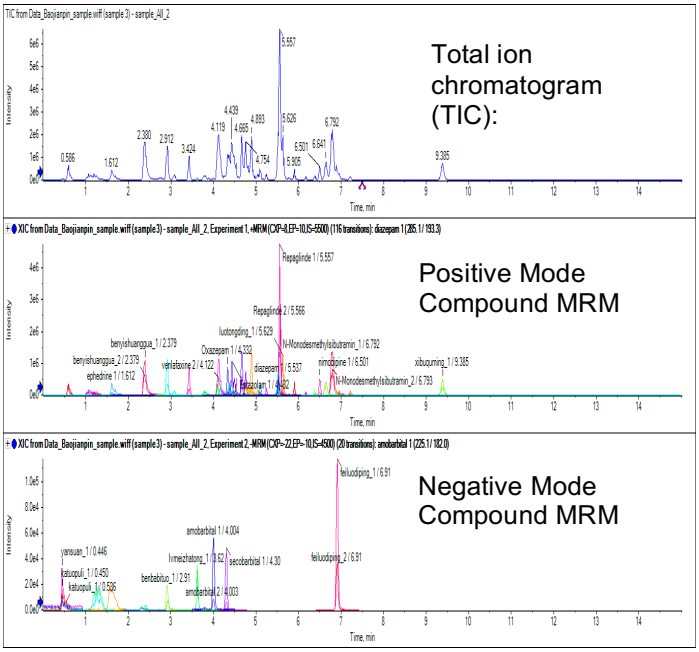
Collision energy: 35±15V

MRM detection window: 60 sec

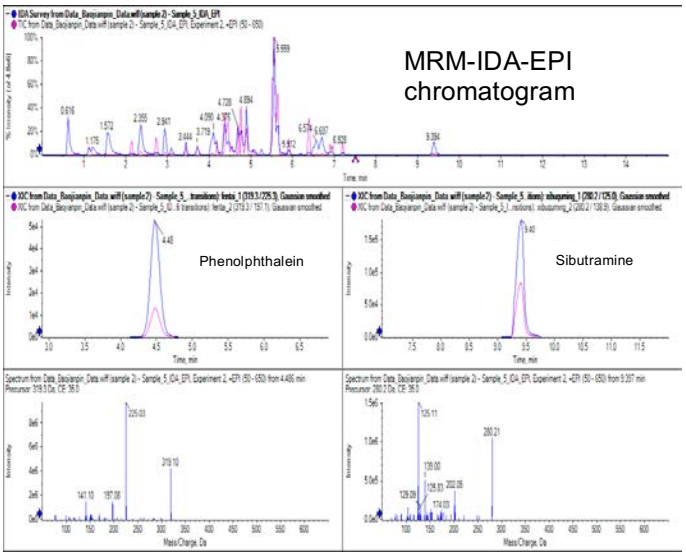
Target Scan Time: 0.25 sec

Experimental results

10 ppb single sample injection of drug mixture, simultaneous positive and negative mode scanning. Extract ion chromatograms as below:

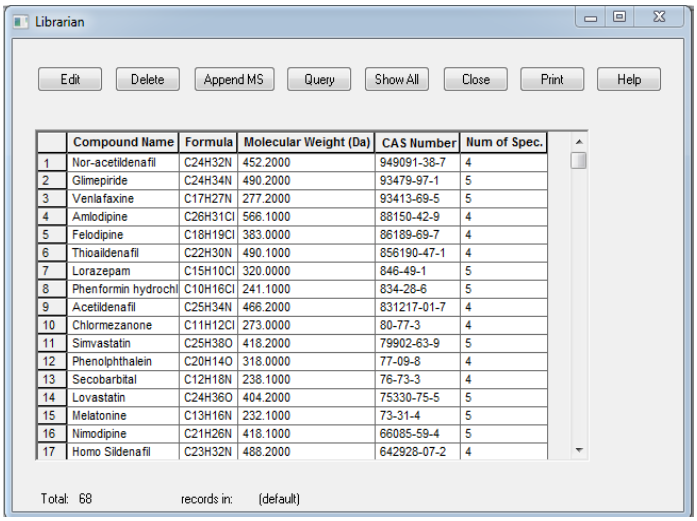


Typical compound MRM extracted ion chromatogram and EPI chromatogram are as follows:



Enhanced secondary fragment （EPI） database

This experimental protocol simultaneously established a database of enhanced secondary fragments (EPI) of 68 drugs. This database includes low, medium, high, and combined energy spectra with large amounts of fragment information and comprehensive validation.



	Compound Name	Formula	Molecular Weight (Da)	CAS Number	Num of Spec.
1	Nor-acetildenafil	C24H32N	452.2000	949091-38-7	4
2	Glimepiride	C24H34N	490.2000	93479-97-1	5
3	Venlafaxine	C17H27N	277.2000	93413-69-5	5
4	Amlodipine	C26H31Cl	566.1000	88150-42-9	4
5	Felodipine	C18H19Cl	383.0000	86189-69-7	4
6	Thioaildenafil	C22H30N	490.1000	856190-47-1	4
7	Lorazepam	C15H10Cl	320.0000	846-49-1	5
8	Phenformin hydrochl	C10H16Cl	241.1000	834-28-6	5
9	Acetildenafil	C25H34N	466.2000	831217-01-7	4
10	Chlormezanone	C11H12Cl	273.0000	80-77-3	4
11	Simvastatin	C25H38O	418.2000	79902-63-9	5
12	Phenolphthalein	C20H14O	318.0000	77-09-8	4
13	Secobarbital	C12H18N	238.1000	76-73-3	4
14	Lovastatin	C24H36O	404.2000	75330-75-5	5
15	Melatonine	C13H16N	232.1000	73-31-4	5
16	Nimodipine	C21H26N	418.1000	66085-59-4	5
17	Homo Sildenafil	C23H32N	488.2000	642928-07-2	4

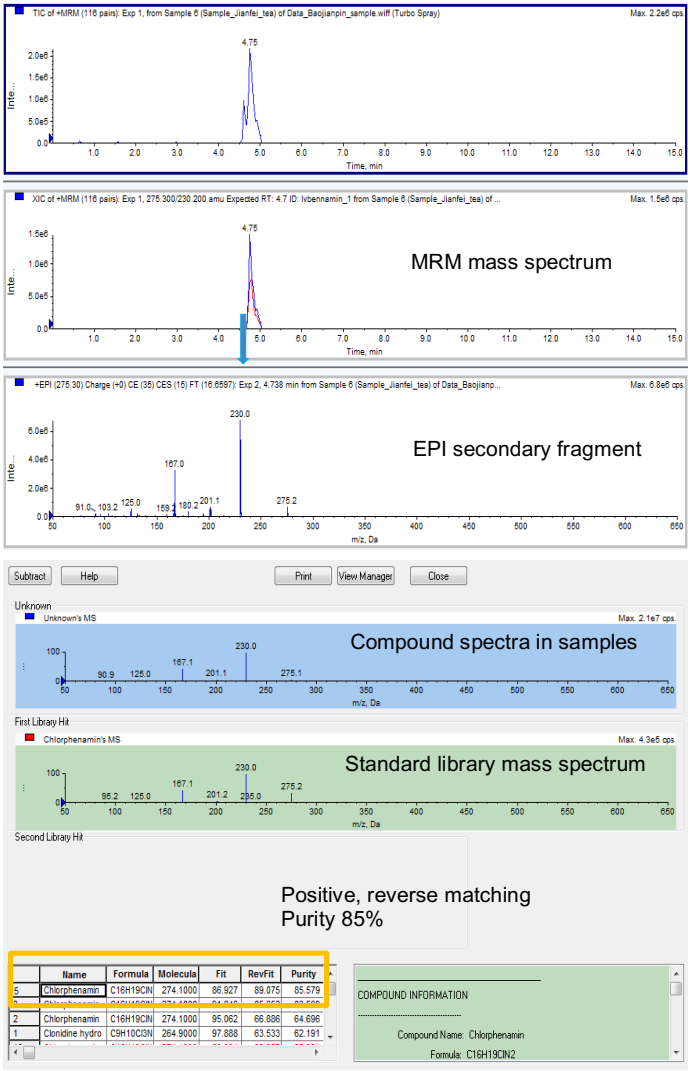
Sample screening results:

Preprocessing: Weight loss tea samples are taken directly after steeping.

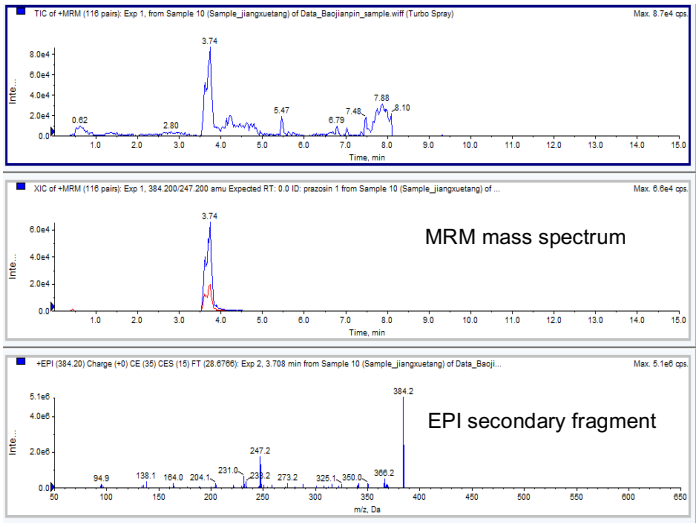
Capsules and tablet drugs: Remove 10mg of capsule contents (or grind tablets to a fine powder), ultrasonicate with 10mL methanol, centrifuge, remove supernatant directly as the sample.

Chlorphenamine has been found in some weight loss tea samples. Right-click library searches based on positive and reverse matching results have verified the presence of chlorphenamine. Chlorphenamine is a drug mainly used for rapid allergy relief.

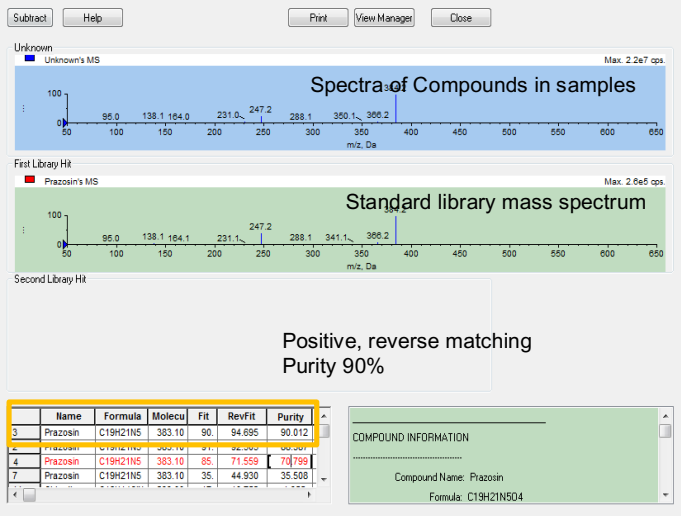
Chlorphenamine has been found in some weight loss tea samples as shown:



Prazosin has been detected in blood sugar-reducing capsules as shown:



Right-click library search based on positive and reverse matching results has verified the presence of prazosin. Prazosin is a prescription drug mainly used for high blood pressure and can cause fainting, dizziness, headache and other adverse reactions.



Conclusions

The SCIEX complex mass spectrometer QTRAP® System's triple quadrupole quantification function and linear ion trap qualification function use just one sample injection to complete both qualitative validation and quantification.

EPI secondary spectral sensitivity is at least 500 times higher than that of traditional MRM scanning. Low, medium, high, and combined energy high sensitivity spectra have more fragments and compensate for the traditional ion trap quality loss effect, so low-mass terminal fragments are abundant. EPI enhanced high-sensitivity secondary spectra effectively help to validate low concentration point detections from complex matrices, which are prone to false positives. This makes results more accurate and reliable. The triple quadrupole complex ion trap mass spectrometer QTRAP's unique MRM-IDA-EPI scanning mode is an effective and comprehensive method for resolving false positives and validating drug detections.

This method is a comprehensive solution based on the SCIEX QTRAP platform for monitoring 68 health products in the 2017 National Food Safety Supervision and Sampling Implementation Guidelines. This protocol includes sample preprocessing, liquid chromatography-mass spectrometry, and secondary validation libraries. It is convenient and quick to use.

Positive ion chemical compound MRM parameters:

No.	Q1	Q3	RT	ID	DP	EP	CE	CXP
1	285.1	193.3	5.55	Diazepam 1	80	10	45	8
	285.1	154.1	5.55	Diazepam 2	80	10	45	8
2	287.2	241.2	4.33	Oxazepam 1	50	10	31	8
	287.2	269.3	4.33	Oxazepam 2	50	10	31	8
3	321.1	275.1	4.48	Lorazepam 1	60	10	30	8
	321.1	303.1	4.48	Lorazepam 2	60	10	30	8
4	295.2	267.3	4.45	Estazolam 1	70	10	34	8
	295.2	205.2	4.45	Estazolam 2	70	10	34	8
5	309.1	281.1	4.66	Alprazolam 1	80	10	33	8
	309.1	274.2	4.66	Alprazolam 2	80	10	33	8
6	343.2	308.2	4.76	Triazolam 1	80	10	36	8
	343.2	315.2	4.76	Triazolam 2	80	10	36	8
7	316.2	270.2	4.54	Clonazepam 1	75	10	36	8
	316.2	214.1	4.54	Clonazepam 2	75	10	49	8
8	267.2	145.2	1.04	Atenolol 1	60	10	38	8
	267.2	190.3	1.04	Atenolol 2	60	10	26	8
9	278.3	58.1	4.04	Venlafaxine 1	40	10	40	8
	278.3	259.9	4.04	Venlafaxine 2	40	10	17	8
10	347.3	315.2	5.25	Nifedipine 1	60	10	12	8
	347.3	271.4	5.25	Nifedipine 2	60	10	16	8
11	361.3	315.1	6.17	Nitrendipine 1	80	10	13	8
	361.3	329.2	6.17	Nitrendipine 2	80	10	20	8
12	419	343.1	6.51	Nimodipine 1	60	10	13	8
	419	359.1	6.51	Nimodipine 2	60	10	22	8
13	232.2	159.3	4.33	Fenflutamine 1	20	10	32	8
	232.2	187.3	4.33	Fenflutamine 2	20	10	20	8
14	446.2	321.2	3.43	Glipizide 1	85	10	20	8
	446.2	103	3.43	Glipizide 2	85	10	62	8
15	453.3	230.2	5.56	Repaglinde 1	100	10	38	8
	453.3	162	5.56	Repaglinde 2	100	10	27	8
16	367.1	170.2	4.39	Glibornuride 1	82	10	24	8
	367.1	152.2	4.39	Glibornuride 2	82	10	27	8
17	206	60.2	2.36	Phenformin hydrochloride 1	80	10	31	8
	206	105	2.36	Phenformin hydrochloride 2	80	10	36	8
18	357.4	193	5.66	Pioglitazone hydrochloride 1	108	10	38	8
	357.4	165	5.66	Pioglitazone hydrochloride 2	108	10	34	8
19	266	125	6.7	N-monodesmethylsibutramin 1	62	10	32	8

	266	138.9	6.7	N-monodesmethylsibutramin 2	62	10	20	8
20	158.1	60.2	1.18	Butyl-biguanide hydrochloride 1	75	10	23	8
	158.1	116.1	1.18	Butyl-biguanide hydrochloride 2	75	10	23	8
21	130.3	60.2	0.58	Metformin hydrochloride 1	45	10	20	8
	130.3	71.2	0.58	Metformin hydrochloride 2	45	10	30	8
22	489.2	312.3	5.56	Vardenafil 1	130	10	53	8
	489.2	151	5.56	Vardenafil 2	130	10	53	8
23	489.2	72.3	5.93	Homo sildenafil 1	130	10	90	8
	489.2	113.3	5.93	Homo sildenafil 2	130	10	41	8
24	467.4	111.1	4.75	Acetildenafil 1	130	10	42	8
	467.4	127.1	4.75	Acetildenafil 2	130	10	42	8
25	505.3	113.3	7.2	Thioaildenafil 1	115	10	44	8
	505.3	327.1	7.2	Thioaildenafil 2	115	10	41	8
26	609.4	195.1	6.93	Reserpine 1	170	10	52	8
	609.4	397.2	6.93	Reserpine 2	170	10	38	8
27	355.9	192	5.64	Tetrahydropalmatine 1	115	10	39	8
	355.9	165	5.64	Tetrahydropalmatine 2	115	10	36	8
28	358.4	135.1	4.88	Rosiglitazone maleate 1	90	10	36	8
	358.4	107.1	4.88	Rosiglitazone maleate 2	90	10	51	8
29	275.3	230.2	4.68	Chlorphenamin 1	60	10	24	8
	275.3	167	4.68	Chlorphenamin 2	60	10	51	8
30	453.2	113.3	4.74	Noracetildenafil 1	130	10	44	8
	453.2	297.3	4.74	Noracetildenafil 2	130	10	53	8
31	460.3	283.1	6.9	Norneosildenafil 1	105	10	48	8
	460.3	299.3	6.9	Norneosildenafil 2	105	10	47	8
32	505.3	99.2	4.83	Hydroxyhomosildenafil 1	108	10	61	8
	505.3	299.2	4.83	Hydroxyhomosildenafil 2	108	10	56	8
33	330.2	181.1	2.72	Sinomenine 1	106	10	46	8
	330.2	239	2.72	Sinomenine 2	106	10	34	8
34	252.2	125	6.55	N,N-didesmethylsibutramin 1	50	10	30	8
	252.2	139	6.55	N,N-didesmethylsibutramin 2	50	10	16	8
35	460.3	283.3	6.39	Pseudovardenafil 1	105	10	49	8
	460.3	299.3	6.39	Pseudovardenafil 2	105	10	52	8
36	280.2	125	9.4	Sibutramine 1	50	10	33	8
	280.2	138.9	9.4	Sibutramine 2	50	10	22	8
37	475.2	100	5.47	Sildenafil 1	130	10	42	8
	475.2	283.1	5.47	Sildenafil 2	130	10	53	8
38	389.3	245	4.1	Zopiclone 1	62	10	23	8
	389.3	217	4.1	Zopiclone 2	62	10	44	8
39	390.1	268.2	4.72	Tadalafil 1	100	10	20	8

	390.1	169.2	4.72	Tadalafil 2	100	10	52	8
40	409.3	238	5.41	Amlodipine 1	116	10	16	8
	409.3	294.2	5.41	Amlodipine 2	116	10	15	8
41	319.3	225.3	4.46	Phenolphthalein 1	90	10	29	8
	319.3	197.1	4.46	Phenolphthalein 2	90	10	41	8
42	528.6	403.2	5.76	Gliquidone 1	98	10	19	8
	528.6	386.3	5.76	Gliquidone 2	98	10	31	8
43	324	110	3.72	Gliclazide 1	95	10	28	8
	324	127.1	3.72	Gliclazide 2	95	10	30	8
44	419.5	199.2	7.78	Simvastatin 1	90	10	18	8
	419.5	243.2	7.78	Simvastatin 2	90	10	19	8
45	306.2	236.2	4.15	Zaleplon 1	96	10	36	8
	306.2	264.2	4.15	Zaleplon 2	96	10	30	8
46	496.5	371.2	4.85	Glibenclamide 1	77	10	22	8
	496.5	171.2	4.85	Glibenclamide 2	77	10	38	8
47	405.5	199.3	7.32	Lovastatin 1	79	10	19	8
	405.5	285.3	7.32	Lovastatin 2	79	10	15	8
48	389.5	240	6.72	Nisoldipine 1	73	10	35	8
	389.5	194.9	6.72	Nisoldipine 2	73	10	30	8
49	230	160	2.1	Clonidine hydrochloride 1	80	10	47	8
	230	145	2.1	Clonidine hydrochloride 2	80	10	51	8
50	233.3	174.1	2.92	Melatonine 1	68	10	18	8
	233.3	158.9	2.92	Melatonine 2	68	10	34	8
51	271.3	155.2	3.07	Tolbutamide 1	71	10	25	8
	271.3	74.1	3.07	Tolbutamide 2	71	10	24	8
52	391.2	169	4.25	Amino tadalafil 1	104	10	45	8
	391.2	268.9	4.25	Amino tadalafil 2	104	10	21	8
53	166.1	148.1	1.55	Ephedrine 1	40	10	18	8
	166.1	133.1	1.55	Ephedrine 2	40	10	26	8
54	491.3	125.9	5.08	Glimepiride 1	50	10	35	8
	491.3	352.1	5.08	Glimepiride 2	50	10	35	8
55	300	283.1	4.6	Chlordiazepoxide 1	80	10	25	8
	300	227.1	4.6	Chlordiazepoxide 2	80	10	25	8
56	326.2	291.4	5.52	Midazolam maleate 1	65	10	37	8
	326.2	244.2	5.52	Midazolam maleate 2	65	10	35	8
57	282.2	236.2	4.38	Nitrazepam 1	70	10	32	8
	282.2	180.2	4.38	Nitrazepam 2	70	10	52	8
58	384.2	247.2	3.74	Prazosin 1	60	10	39	8
	384.2	138.2	3.74	Prazosin 2	60	10	43	8

Anion Compound Parameters

No.	Q1	Q3	RT	ID	DP	EP	CE	CXP
59	183.1	140	1.48	Barbital 1	-50	-10	-16	-22
	183.1	95.9	1.48	Barbital 2	-50	-10	-20	-22
60	122.2	77.8	0.43	Nicotinic acid 1	-45	-10	-16	-22
	122.2	122.2	0.43	Nicotinic acid 2	-45	-10	-10	-22
61	230.9	144.2	2.88	Phenobarbital 1	-57	-10	-22	-22
	230.9	85	2.88	Phenobarbital 2	-57	-10	-16	-22
62	225.1	182	4	Amobarbital 1	-30	-10	-17	-22
	225.1	85	4	Amobarbital 2	-30	-10	-19	-22
63	237.1	194	4.29	Secobarbital 1	-40	-10	-17	-22
	237.1	85	4.29	Secobarbital 2	-40	-10	-17	-22
64	295.9	268.9	1.16	Hydrochlorothiazide 1	-101	-10	-26	-22
	295.9	204.9	1.16	Hydrochlorothiazide 2	-101	-10	-32	-22
65	329	204.9	2.38	Furosemide 1	-109	-10	-26	-22
	329	284.9	2.38	Furosemide 2	-109	-10	-21	-22
66	382.1	144.8	6.92	Felodipine 1	-69	-10	-14	-22
	382.1	236	6.92	Felodipine 2	-69	-10	-20	-22
67	216	182	0.44	Captopril 1	-58	-10	-17	-22
	216	113.8	0.44	Captopril 2	-58	-10	-16	-22
68	271.9	179.8	3.62	Chlormezanone 1	-51	-10	-21	-22
	271.9	208	3.62	Chlormezanone 2	-51	-10	-16	-22

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