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Adulteration of Drugs in Food - Glucocorticoids, Anorexics and Hypnotic-Sedatives (I)

FANG-YING TSAI[1]*, NEN-TNU WEI[1], CHEN-YUAN WU[1], YU-PE CHEN[1] AND KUO-CHING WEN[2]

[1.] *Buruea of Food and Drug Analysis, 161-2 Kunyang St., Nangang District, Taipei City 115, Taiwan (R.O.C.)*

[2.] *Department of Cosmeceutics, China Medical University, 91 Hsueh-Shih Rd., North Section, Taichung City 404, Taiwan (R.O.C.)*

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ABSTRACT

In this study, we evaluated the adulteration of glucocorticoids, anorexics as well as hypnotic-sedatives in food products that claim to have medicinal effects and/or are assigned to specific category of drugs. The possible addition of drugs in food was also investigated. The TLC method was established to identify the components of each drug category using UV spectra obtained from TLC analysis. LC/MS and GC/MS were used to determine the optimal detection concentration of each drug by mixing the three placebo foods with drugs in 3 categories respectively.

This paper shows the capability of the analytical method tested on the simulated food samples. Results showed that the optimal detection concentration of the three drug categories obtained are as follows: the concentration range of 7 ingredients of glucocorticoids is from 1.25 mg/g prednisolone to 2.5 mg/g triamcinolone; 6 ingredients of anorexics is from 1.25 mg/g diethylpropion to 2.5 mg/g phentermine, 12 drugs of the third category is from 0.1 mg/g methaqualone to 3 barbitals (0.4 mg/g).

The Standard Operating Procedures (SOPs) for the screening method, the UV spectra and the concentration of each drug offered as references for the qualitative method for the analysis.

Key words: food, glucocorticoids, anorexics, hypnotic-sedatives, TLC, UV, optimal detection concentration

INTRODUCTION

Most commercialized food products are made from single animal/vegetable, combinations of animals/vegetables, or complicated recipes. The general population considers these ingredients with no herbal or drug ingredients to be without side effects, and has the potential to strengthen the physical body as claimed in many advertisements. The public is under the impression that taking more of these food products would not cause additional harm. Due to the need for available medicines with effective curing abilities and particularly with little or no side effects, some consumers would turn to purchase these claimed harmless, edible and healthier food. Individual direct-sale merchandisers or distributors continue to allure consumers via widespread advertisements. Statistics from recent analysis data had shown that[(1)]: (1) about 1-2% of drug-contamination was found to be from products with no labeling of manufacturer, manufacturing date, expiry date or were not the original package, (2) drug adulteration in liver-protecting foods contained considerable amounts of added drugs, and (3) diazepam adulteration in liver-protecting and sedative foods should be further investigated.

There are many analytical methods published, e.g. TLC[(2-5,7)] in qualitatively analyzing diethylpropion, methamphetamine, phentermine, phenylpropanolamine, dexamethasone, phenobarbital, caffeine, prednisolone and diazepam; or GC/MS[(6)] in qualitatively analyzing alprazolam, bromazepam, diazepam, flunitrazepam, flurazepam and triazolam; or HPLC[(8)] in quantitatively analyzing dexamethasone, prednisolone, caffeine and diazepam from herbal products; or capillary electrophoresis[(9)] in qualitatively analyzing diazepam, dexamethasone, prednisolone and caffeine. Due to the complexity of food resources, unknown food ingredients or the composition of vegetables and animal parts, the drugs were also difficult to ascertain. In this study, we applied 3 different developing solvents, in combination with chromogenic analysis and UV spectrophotometry, using TLC to separate the drug components and compare the results with standard drug solution in screening, followed by identification with other instruments, such as GC/MS and LC/MS. Three categories of drugs with potential medicinal effects and possible adulteration sources are listed as follows:

I. Glucocorticoids: betamethasone, cortisone acetate, prednisolone, dexamethasone, methylprednisolone and triamcinolone.

II. Anorexics: diethylpropion, fenfluramine, methamphetamine, phentermine, phenylpropanolamine, and caffeine.

III. Hypnotic-Sedatives: alprazolam, bromazepam, diazepam, flurazepam, flunitrazepam, methaqualone, nitrazepam, oxazolam, barbital, phenobarbital, triazolam, and secobarbital sodium.

* Author for correspondence. Tel: 886-2-26531220;
Fax: 886-2-26531213; E-mail: fangying@nlfd.gov.tw

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MATERIALS AND METHODS

I. *Instruments and Materials*

Several instruments were used in this study, including a UV lamp (with 254 and 366 nm in wavelength), LC/MS, GC/MS (Micromass Trio-2000/HP 5890), Spectrophotometer (HITACHI UV/VIS U-320), an oven and a sonicator.

II. *Comparative Standards of Drug Components*

Betamethasone, cortisone acetate, prednisolone, dexamethasone, methylprednisolone, triamcinolone, diethylpropion, fenfluramine, methamphetamine, phentermine, phenylpropanolamine, caffeine, alprazolam, bromazepam, diazepam, flunitrazepam, flurazepam, methaqualone, nitrazepam, oxazolam, barbital, phenobarbital, triazolam, and secobarbital sodium were purchased from Sigma Com.

III. *Reagents and Solvents*

Ethyl acetate, ether, acetonitrile, chloroform, acetone, methanol, methyl benzene, and ethanol were HPLC grade; while 95% ethanol, sulfuric acid, ammonium acetate, cobalt chloride, mercury nitrate, bismuth nitrite, potassium iodide, copper sulfate, sodium hydroxide, ninhydrin, vanillin, tetrazolium blue, pyridine were in reagent grade.

IV. *Analytical Conditions*

(I) *LC/MS conditions*

1. Instrument: HP 1100 LC/MS ESI-Negative Mode.
2. Chromatographic column; Luna 3U C18(2), 100 mm × 2 mm × 3 μm.
3. Mobile phase: CH_3CN/20 mM ammonium acetate (35/65) was maintained for 3 min, and then the concentration was changed gradiently to 20/80 in 13 min.
4. Flow rate: 0.2 mL/min.
5. Injection volume: 1 μL.
6. Interphase of spectrophotometer: API-ES, in polarity of negative or positive mode; fragmentor voltage: 100V; nebulizer gas pressure: 30 psi; drying gas flow: 8 L/min; drying gas temperature: 350˚C; capillary voltage: 3500V.

(II) *GC/MS conditions*

1. Instrument: Micromass Trio-2000 (HP5890).
2. Chromatographic column: Rtx-5MS (30 m × 0.25 mm × 0.25 μm).
3. Temperature of chromatographic column: 100˚C for 5 min, and then elevated, in a rate of 20˚C/min, to 270˚C for 20 min.
4. Injection port temperature: 250˚C.
5. Interface temperature: 250˚C.
6. Flow rate of helium: 1 mL/min.
7. Injection volume: 1 μL.
8. Spectrophotometer: Quadrupole as the detector; detected by electron impact (EI^+): 70 eV; filament emission current: 200 μV; source temperature: 180˚C; solvent delay: 4 min.

V. *Developing Solvents*

1. Chloroform: methanol (9:1, v/v).
2. Chloroform: ethyl acetate (1:1, v/v).
3. Ethyl acetate: ether (4:1, v/v).
4. Methanol: ammonia solution (100:1.5, v/v).
5. Ethyl acetate: methanol:ammonia solution (17:2:1, v/v).
6. Chloroform: acetone (4:1, v/v).

VI. *Preparation and Usage of Chromogens*

(I) Dragendorff's spray reagent[10]: Took 2 g of bismuth nitrite and mix it with 25 mL of acetic acid and 100 mL of water to prepare solution A. Forty gram of potassium iodide was, dissolved in 100 mL of water to prepare solution B. Solution A and B were mixed in equal volumes (freshly prepared).

(II) Fifty % sulfuric acid-ethanol spray reagent[11]: Equal volumes of sulfuric acid and ethanol were mixed together.

(III) Zwikker's reagent[12]: Ten grams 10 g of copper sulfate was transfered to a 100-mL volumetric flask and dissolved in 100 mL of water to prepare solution A. Forty mL of solution A was mixed with 10 mL of pyridine and then water was added to prepare a total volume of 100 mL.

(IV) Marquis reagent: Ten mL of formaldehyde was mixed with 90 mL of sulfuric acid.

(V) Tetrazolium Blue spray reagent: Transfer 0.5 g of tetrazolium Blue to a 100-mL volumetric flask and dissolve with 100 mL of methanol to make solution A. Five of mL solution A was diluted with 2 M sodium hydroxide-methanol to prepare a total volume of 50 mL.

(VI) Vanillin-sulfuric acid: One gram of vanillin was transfered to a volumetric flask and dissolved in 100 mL of sulfuric acid.

(VII) Ninhydrin spray reagent: Ninhydrin (0.5 g) was transfered to a volumetric flask and dissolve in l00 mL of acetone.

VII. *Preparation of Standard Solutions and Sample Solution*

(I) *Preparation of glucocorticoids standard solutions*

Ten mg of glucocorticoids, including betamethasone, cortisone acetate, methylprednisolone, prednisolone, prednisone, dexamethasone, and triamcinolone were weighed accurately and respectively, and then transferred into a 10-mL volumetric flask respectively. An aliquot of methanol

was added to make a total volume of 10 mL, as the standard solution.

(II) *Preparation of anorexics standard solutions*

Ten mg of diethylpropion, fenfluramine, and caffeine, 20 mg of methamphetamine, and phentermine, and 40 mg of phenylpropanolamine were weighed accurately and respectively, and then placed in a 10-mL volumetric flask respectively. An aliquot of methanol was added to make a total volume of 10 mL, as the standard solution.

(III) *Preparation of hypnotic-sedatives standard solutions*

Ten mg of hypnotic-sedatives, including alprazolam, diazepam, bromazepam, flunitrazepam, flurazepam, methaqualone, nitrazepam, oxazolam, barbital, phenobarbital, triazolam, and secobarbital sodium were weighed accurately and respectively, and then transferred into a 10-mL volumetric flask respectively. An aliquot of methanol was added to make total volume of 10 mL, as the standard solution.

(IV) *Preparation of sample solution*

Accurately weighed 1 g of the finely sample powder was transferred into a screw-capped tube, and dissolved in an aliquot of methanol. After sonicating for 5 min, and centrifugation, the supernatant was collected as the sample solution.

VIII. *Optimal Detection Concentrations*

(I) *Optimal detection concentrations in liquid chromato graphy*

Methanol was used as the diluent. Ultra-Trim (Healtheries of NZ Ltd.), Alisin Capsules (Excellent Corporation) and Feslim Tablets (Excellent Corporation) were chosen as the blank samples. Glucocorticoids, (e.g. betamethasone, cortisone acetate, methylprednisolone, prednisone, prednisolone, dexamethasone, triamcinolone), anorexics, (e.g. diethylpropion, fenfluramine, methamphetamine, phentermine, phenylpropanolamine, caffeine), and hypnotic-sedatives (e.g. alprazolam, bromazepam, diazepam, flurazepam, flunitrazepam, methaqualone, nitrazepam, oxazolam, barbital, phenobarbital, triazolam, secobarbital sodium) were spiked individually for the determination of optimal detection concentrations.

(II) *Preparation of glucocorticoids-adulterated testing sample solutions*

Accurately weighed 1 g of the finely sampled powder was transferred into a screw-capped tube. Ten mg of individual glucocorticoids standard solution, including betamethasone, cortisone acetate, methylprednisolone, prednisolone, prednisone, dexamethasone, and triamcinolone, was weighed accurately and dissolved in 5 mL of methanol. After sonicating for 5 min, and centrifugation, the supernatant was collected as the sample solution.

(III) *Preparation of anorexics-adulterated testing sample solutions*

Accurately weighed 1 g of the finely sampled powder was transferred into a screw-capped tube. Individual anorexics standard solution, including 10 mg of diethylpropion, fenfluramine, and caffeine, 20 mg of methamphetamine, and phentermine, 40 mg of phenylpropanolamine, was weighed accurately and then placed in a 10-mL volumetric flask. An aliquot of methanol was added to dissolve the material. After sonicating for 5 min, and centrifugation, the supernatant was collected and volumetrically adjusted as the standard solution.

(IV) *Preparation of hypnotic-sedatives-adulterated testing sample solutions*

Accurately weighed 1 g of the finely sample powder was transferred into a screw-capped tube. Ten mg of individual hypnotic-sedatives standard solution, including alprazolam, bromazepam, diazepam, flurazepam, flunitrazepam, methaqualone, nitrazepam, oxazolam, barbital, phenobarbital, triazolam, secobarbital sodium, was weighed accurately and dissolved in 5 mL of methanol. After sonicating for 15 min to dissolve completely, and centrifugation, the supernatant was collected as the sample solution for the determination of optimal detection concentration in GC/MS and LC/MS systems.

(V) *Determination of the optimal detection concentration in GC/MS and LC/MS*

Accurately weighed 1 g of the finely testing sample powder was transferred into a screw-capped tube. Individually weighted 20 mg, 10 mg, 5 mg, 2.5 mg, 1.25 mg, 0.625 mg, 0.31 mg, and 0.155 mg of the abovementioned standard solutions and placed into the screw-capped tube. After addition of methanol (5 mL), sonication for 15 min, and centrifugation, the supernatant was under analysis. The concentration higher than the undetectable level is defined as the optimal detection concentration.

IX. *UV Spectrophotometry*

The standard solutions spiked with different drug categories were spread with a thin-layer chromatography (Kieselgel $60F_{254}$, 20 × 20 cm, 0.25mm; E. Merck, Darmstadt) and examined under UV 254 nm. The spot was scratched and dissolved in methanol. After centrifugation and filtration, the filtrate was examined with a UV spectrophotometer. The UV absorption spectra were used as the interfering reference of blank test sample.

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RESULTS

We analyzed 25 drug-adulterated foods containing drugs from 3 categories, including glucocorticoids, anorexics, and hypnotic-sedatives, by TLC method, according to claimed medicinal effect or by assignation. To sum up, there were 25 TLC methods developed. The individual UV 254nm, R_f value, detection and chromogenic reaction from TLC analysis of glucocorticoids, anorexics, and hypnotic-sedatives are shown in Table 1-3. The R_f value of glucocorticoids, anorexics, and hypnotic-sedatives were in the range of 0.06~0.91, 0.05~0.92, 0.17~ 0.92,

Table 1. The R_f value of TLC and spray reagent used to analyze the adulterated glucocorticoids in foods

Compound	Analytic method						
	Solvent and r_f value			UV 254 nm	Reagent and color		
	1	2	3		B (366nm)	E	F
Betamethasone	0.30	0.22	0.83	+	Gray (light pink)	Violet	Gray
Cortisone acetate	0.64	0.48	0.91	+	Light brown (pale yellow)	Violet	Light gray
Dexamethasone	0.23	0.20	0.81	+	Gray (light pink)	Violet	Gray
Methylprednisolone	0.18	0.13	0.69	+	Brown (cinnamon)	Violet	Brown
Prednisolone	0.16	0.13	0.64	+	Brown (yellow)	Violet	Brown
Prednisone	0.27	0.20	0.65	+	Gray (cinnamon)	Violet	Light brown
Triamcinolone	0.07	0.06	0.29	+	Gray (pink)	Violet	Light gray

Solvent 1: chloroform/ethanol (9/1). Solvent 2: chloroform/ethyl acetate (1/1).
Solvent 3: ethyl acetate/ether (4/1). B: 50% sulfuric acid-ethanol solution.
E: Tetrazonlium-alkali/methanol spray reagent. F: Vanillin-sulfuric acid spray reagent.
+: Spot can be visible at wavelength 254 nm.

Table 2. The R_f value of TLC and spray reagent used to analyze the adulterated anorexics in foods

Compound	Analytic method						
	Solvent and R_f value			UV 254 nm	Reagent and color		
	1	2	3		A	B	G
Betamethasone	0.30	0.22	0.83	+	Gray (light pink)	Violet	Gray
Caffeine	0.86	0.53	0.17	+	--	Orange	--
Diethylpropion	0.80	0.54	0.92	+	Orange	Orange	Vermeil
Fenfluramine	0.30	0.29	0.84	+	Orange	Orange	Puce
Methamphetamine	0.14	0.17	0.63	+	Orange	Orange	Light purple
Phentermine	0.15	0.25	0.71	+	Orange	Orange	Light pink
Phenylpropanolamine	0.05	0.30	0.55	+	Orange	Orange	Dark purple

Solvent 1: chloroform/ethanol (9/1). Solvent 2: methanol/ammonia solution (100/1.5).
Solvent 3: ethyl acetate/methanol/ammonia solution (17/2/1). A: Dragendorff's spray reagent.
B: 50% sulfuric acid-ethanol solution. G: Ninhydrin spray reagent.
--: No color occurred after spray the reagent. +: Spot can be visible at wavelength 254 nm.

Table 3. The R_f value of TLC and spray reagent used to analyze the adulterated hypnotic-sedatives in foods

Compound	Analytic method						
	Solvent and R_f value			UV 254 nm	Reagent and color		
	1	2	3		A	C	D (366 nm)
Alprazolam	0.75	0.2	0.78	+	Orange	--	--(Brown)
Bromazepam	0,67	0.28	0.11	+	Orange	--	Yellow(brown)
Diazepam	0.81	0.75	0.83	+	Orange	--	Orange(brown)
Flurazepam	0.6	0.36	0.87	+	Orange	--	Pink (brown)
Flunitrazepam	0.80	0.75	0.9	+	Orange	--	Orange(teal blue)
Methaqualone	0.92	0.83	0.91	+	Orange	--	--(Dark blue)
Nitrazepam	0.63	0.36	0.87	+	Orange	--	Orange (vermeil)
Oxazolam	0.6	0.68 (Tailing)	0.49	+	Orange	--	--(Blue)
Barbital	0.54	0.48	0.48	+	Orange	Pink	--(Light brown)
Phenobarbital	0.58	0.51	0.22	+	Orange	Pink	--(Brown)
Secobarbital Sodium	0.74	0.68	0.89	+	Orange	Pink	--(Violet)
Triazolam	0.64	0.18	0.17	+	Orange	--	--(Gray)

Solvent 1: chloroform/ethanol (9/1). Solvent 2: chloroform/acetone (4/1).
Solvent 3: ethyl acetate/ether (4/1). A: Dragendorff's spray reagent.
C: Zwikker's reagent. D: Marquis reagent.
--: No color occurred after spray the reagent. +: Spot can be visible at wavelength 254 nm.

respectively. The 9-time R_f values were within the average ±30% restriction limit, when standard solution and testing solution of the 3 sample categories were spotted 3 times in chloroform/ethyl acetate (1/1, v/v), methanol/ammonia solution (100/1.5, v/v), and chloroform/acetone (4/1, v/v) (Table 4-6). In the spectrophotometric analysis of drug-

Table 4. The quality control chart of glucocorticoidal Rf value

	Average R_f	R_f range	Standard deviation (SD)	Control limit (±30%)
Betamethasone	0.22	0.19~0.24	0.02	0.15-0.28
Cortisone Acetate	0.28	0.47~0.50	0.02	0.44-0.53
Dexamethasone	0.20	0.19~0.20	0.01	0.18-0.21
Methylprednisolone	0.13	0.12~0.14	0.01	0.10-0.16
Prednisolone	0.12	0.12~0.13	0.00	0.11-0.13
Prednisone	0.19	0.19~0.20	0.01	0.18-0.21
Triamcinolone	0.04	0.03~0.04	0.00	0.03-0.05

Solvent: chloroform/ethyl acetate (1/1).

Table 5. The quality control chart of Anorexics R_f Value

	Average R_f	R_f range	Standard deviation (SD)	Control limit (±30%)
Caffeine	0.56	0.47~0.62	0.06	0.38-0.74
Diethylpropion	0.51	0.39~0.61	0.07	0.30-0.72
Methamphetamine	0.15	0.11~0.23	0.04	0.027-0.273
Fenfluramine	0.21	0.16~0.34	0.06	0.042-0.378
Phenylpropanolamine	0.26	0.18~0.37	0.07	0.06-0.46
Phentermine	0.23	0.15~0.32	0.05	0.08-0.38

Solvent: methanol/ammonia solution (100/1.5).

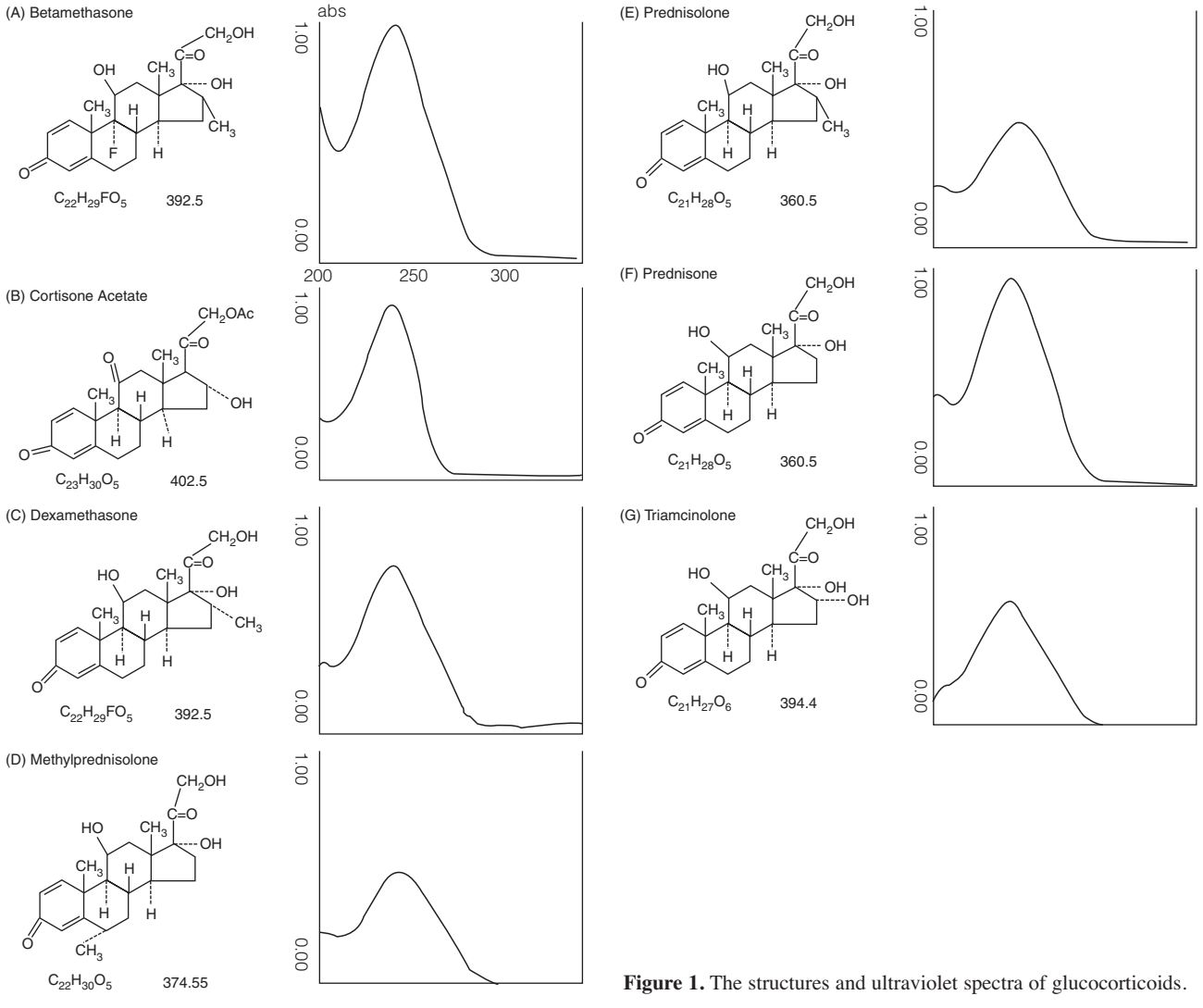

Figure 1. The structures and ultraviolet spectra of glucocorticoids.

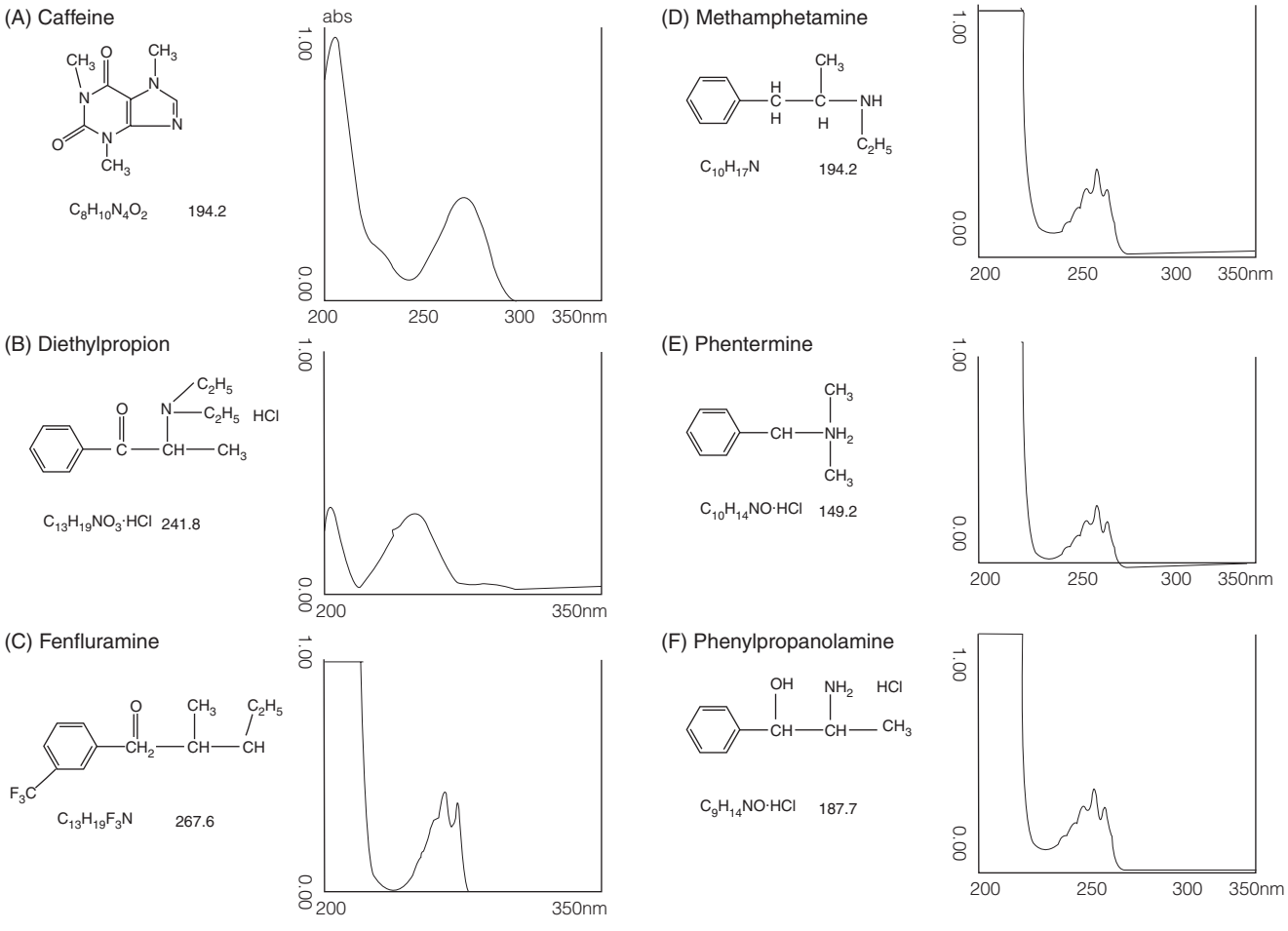

Figure 2. The structures and ultraviolet spectra of anorexics.

Table 6. The quality control chart of hyptonic-sedatives R_f value

	Average R_f	R_f range	Standard deviation (SD)	Control limit (±30%)
Alprazolam	0.16	0.13-0.18	0.017	0.11-0.21
Bromazepam	0.23	0.20-0.28	0.006	0.17-0.28
Diazepam	0.73	0.63-0.86	0.062	0.54-0.91
Flurazepam	0.088	0.07-0.11	0.01	0.06-0.12
Flunitrazepam	0.73	0.65-0.84	0.066	0.53-0.93
Methaqualone	0.76	0.70-0.82	0.032	0.66-0.87
Nitrazepam	0.56	0.73-0.69	0.071	0.35-0.77

Solvent: chloroform/acetone (4/1)

adulterated foods, the 25 absorption spectra were illustrated in Figures 1-3, and the UV absorption wavelengths were shown in Tables 7-9. The LC/MS spectra of 7 glucocorticoids-adulterated foods were illustrated in Figures 5-7. The GC/MS spectra of 6 anorexics-adulterated foods and 12 hypnotic-sedatives-adulterated foods were shown in Figure 8 and 9. We completed the optimal detection concentration analyses of 7 different glucocorticoids in LC/MS by injecting 1 μL of testing sample solution; which included 2.5 mg/g betamethasone, 2.5 mg/g cortisone acetate, 2.5 mg/g dexamethasone, 1.25 mg/g methylprednisolone, 1.25 mg/g prednisolone, 2.5 mg/g prednisone and 2.5 mg/g triamcinolone. The GC/MS analyses of 6 different anorexics, 1.25 mg/g including diethylpropion, 1.25 mg/g caffeine, 2.5 mg/g fenfluramine, 2.5 mg/g methamphetamine, 2.0 mg/g phenylpropanolamine and 2.5

Table 7. The MS fragment (*m/z*) and the UV absorption wavelength of glucocorticoids

Compound	Molecular fragment (*m/z*)	Retention time (min)	Ultraviolet absorptive wavelength (nm)
Betamethasone	391.1 (392.5)	5.30	240
Cortisone acetate	401.1 (402.5)	11.12	240
Dexamethasone	391.1 (392.4)	5.58	240
Methylprednisolone	373 (374)	4.64	240
Prednisolone	359 (360.4)	3.08	240
Prednisone	357 (358.4)	3.29	240
Triamcinolone	393.1 (394.4)	2.13	240

Table 8. The MS fragment (*m/z*) and the UV absorptive wavelength of anorexics

Compound	Molecular fragment (m/z)	Retention time (min)	Ultraviolet absorptive wavelength (nm)
Diethylpropion	100; 44; 72; 101; 77; 56; 42; 105	10.16	248
Fenfluramine	72; 44; 159; 73; 82; 42; 109; 56	7.65	264; 271
Methamphetamine	44; 91; 77; 105; 51; 134	7.92	252; 258; 263
Phentermine	58; 91; 42; 41; 134; 65; 59; 40	6.67	252; 257; 263
Phenylpropanolamine	44; 77; 79; 45; 42; 107; 105	9.07	251; 258; 263
Caffeine	194; 109; 42; 41; 136; 65; 59; 40	12.4	272

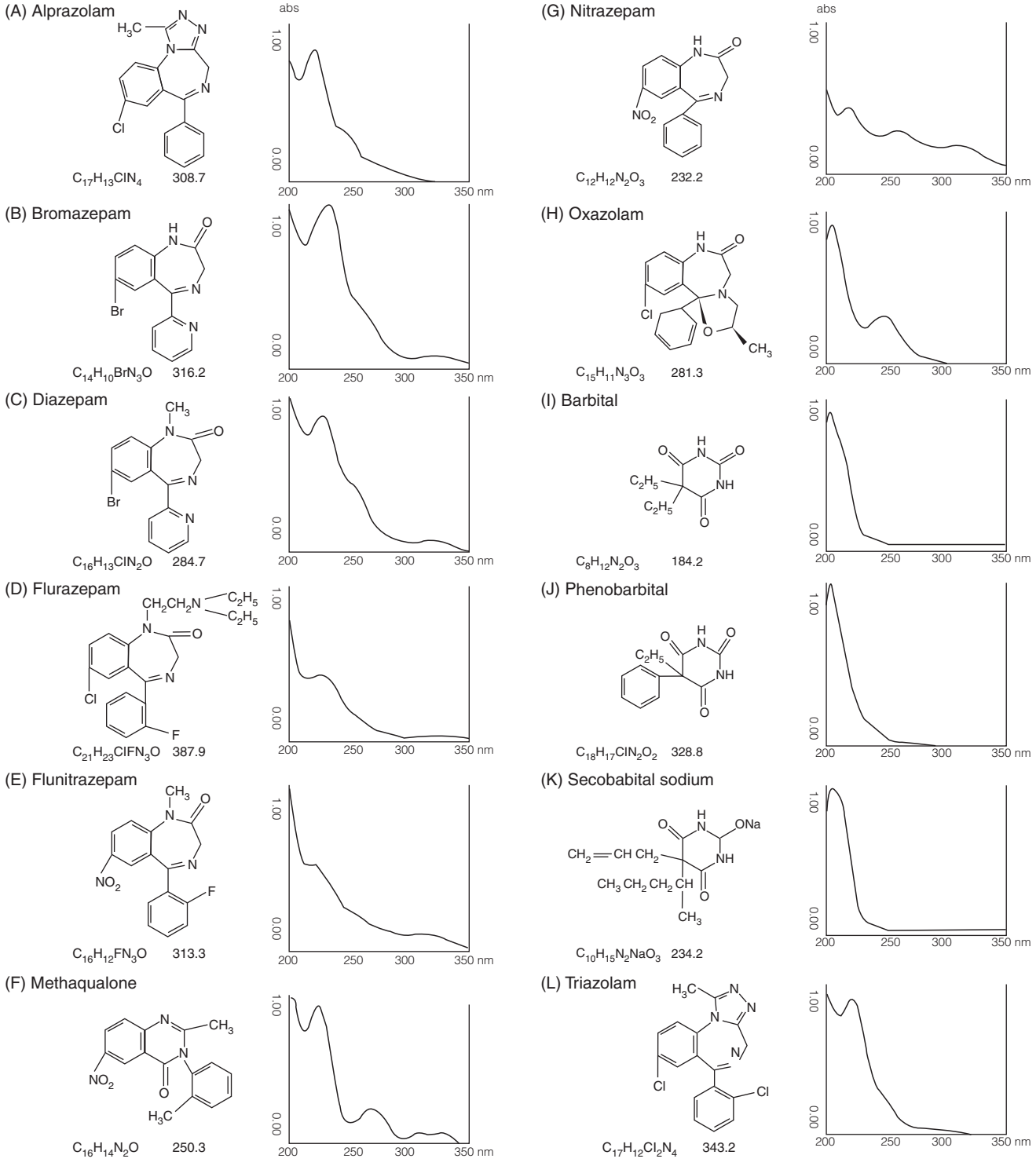

Figure 3. The structures and ultraviolet spectra of hyptonic-sedatives.

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Table 9. The MS fragment (*m/z*) and the UV absorptive wavelength of hypnotic-sedatives

Compound	Molecular fragment (m/z)	Retention time (min)	Ultraviolet absorptive wavelength (nm)
Alprazolam	279; 204; 308; 273; 51; 77; 137	23.68	219
Bromazepam	236; 317; 315; 288; 316; 286; 208	17.95	323; 232
Diazepam	256; 283; 284; 285; 257; 255; 258; 286	15.87	315; 230
Flurazepam	86; 87; 99; 58; 84; 387; 315; 67	18.87	307; 221
Flunitrazeapm	285; 312; 313; 286; 266; 238; 294; 284	17.55	317; 228
Methaqualone	235; 250; 233; 91; 217; 76; 132	14.08	316; 304 265; 225
Nitrazepam	253; 280; 281; 206; 234; 252; 254; 264	19.05	307; 259; 218
Oxazolam	251; 70; 253; 105; 202; 254	16.77	255
Barbital	156; 141; 112; 98; 55; 69; 83	10.29	---
Phenobarbital	204; 117; 232; 161; 146; 77; 103	13.09	---
Secobarbital Sodium	141; 156; 41; 57; 39; 98; 157; 47	12.05	---[a]
Triazolam	313; 238; 342; 315; 75; 344; 239; 137	24.42	219

[a]No absorption at ultraviolet spectra.

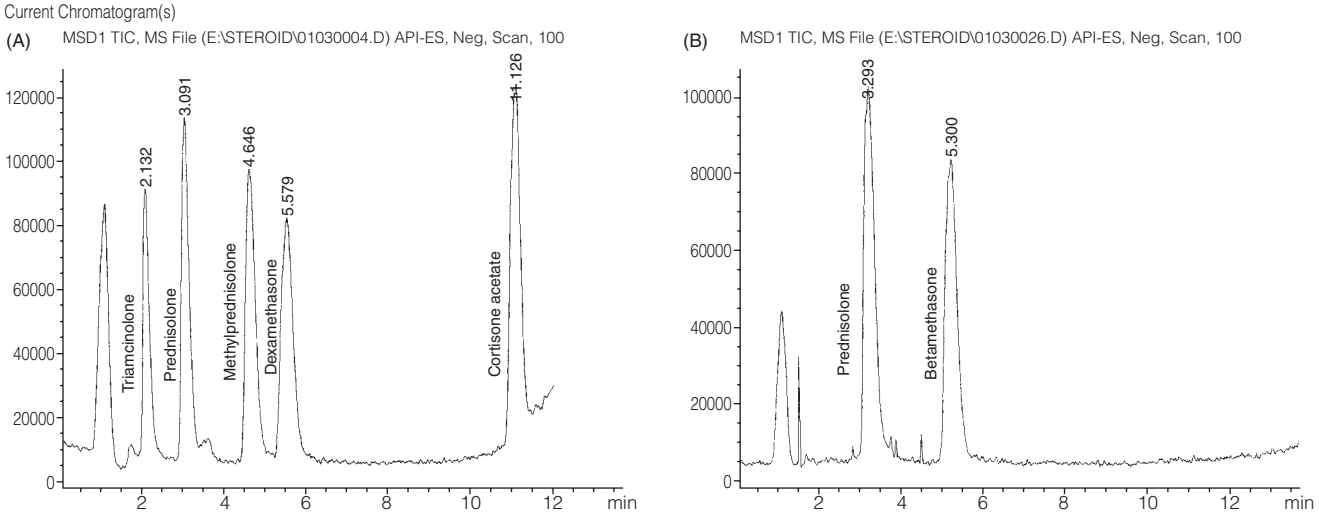

Figure 4. The LC/MS spectra of glucocorticoidal standards.

mg/g phentermine, by injecting 1 μL testing sample solution were accomplished. We also had done GC/MS analyses of 12 different hypnotic-sedatives, including 0.2 mg/g alprazolam, 0.2 mg/g bromazepam, 0.2 mg/g diazepam, 0.2 mg/g flunitrazepam, 0.2 mg/g flunitrazepam, 0.1 mg/g methaqualone, 0.2 mg/g nitrazepam, 0.3 mg/g oxazolam, 0.4 mg/g barbital, 0.4mg/g phenobarbital, 0.4 mg/g secobarbital sodium and 0.2 mg/g triazolam.

DISCUSSION

In this study, we evaluated the efficiency of 3 solvents used in TLC as the developing solutions, including basic and neutral solvents with different polarity, for the identification of 3 categories of glucocorticoids that might be found in food. Most of the time, the products with drug fortification are intended for eating or drinking. Most of the drugs added into food usually have good water solubility, i.e., dissolve easily in polar solvents. We thus used methanol to dissolve drug components in the samples and analyzed the results with TLC and UV spectrophotometry. When compared with standard samples, the contaminated food products were screened and confirmed by $UV_{254\ nm}$, $UV_{366\ nm}$, chromogenic analysis, and UV absorption spectra. The optimal R_f values for each drug component in spreading solvent were within 0.2~0.8[13]. Most of the spreading solvents can fulfill the requirements. For glucocorticoids, the least desired resolution was observed in triamcinolone, with R_f value < 0.1; the second worst were prednisolone and methylprednisolone, with R_f value < 0. 2; only one developing solvent led to better resolution. For anorexics, methamphetamine obtained the least desirable resolution, with R_f value < 0.2 in 2 spreading solvents; the second were phentermine, with R_f value < 0.2 in one spreading solvent, and phenylpropanolamine, with R_f value < 0.1 in one spreading solvent. For hypnotic-sedatives, only triazolam was found with R_f value < 0.2. We also observed that some components, e.g. oxazolam and methylprednisolone, shall be freshly prepared, because if set aside for a while, the components would dissociate into several different spots in TLC. In conclusion, the samples should be analyzed together with the

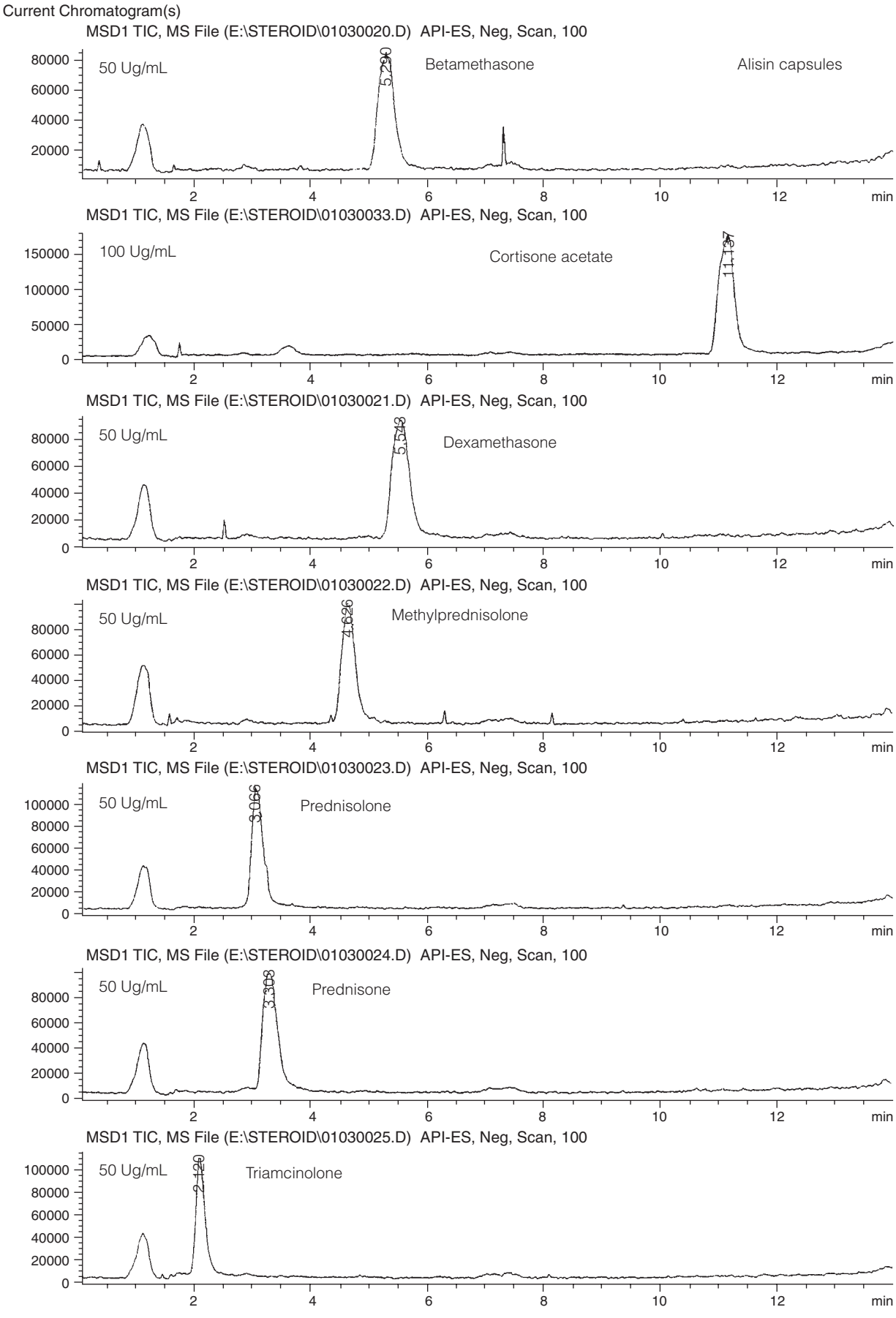

Figure 5. The LC/MS spectra of the spiked glucocorticoids in Alisin capsules.

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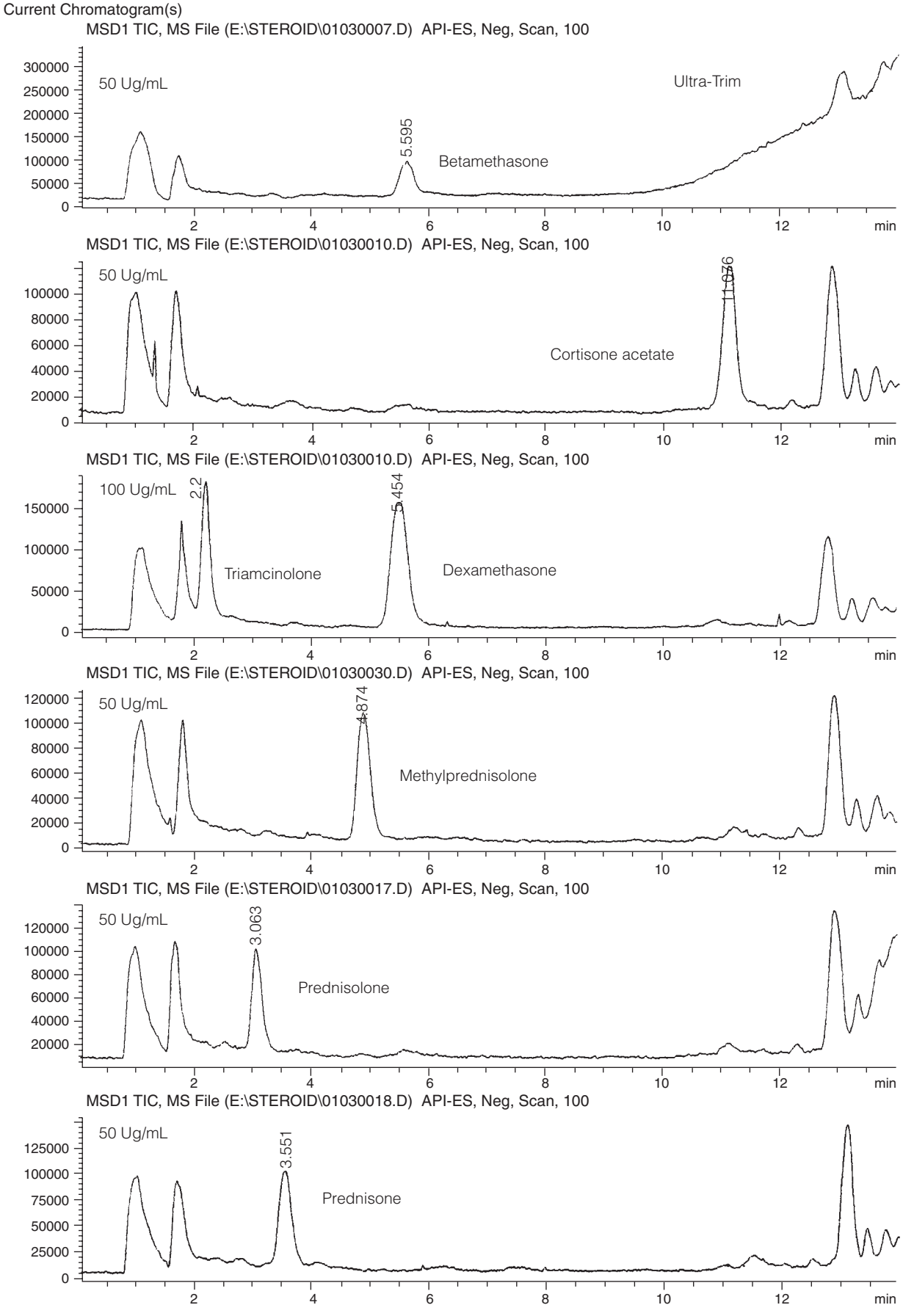

Figure 6. The LC/MS spectra of the spiked glucocorticoids in Ultra-trim powder.

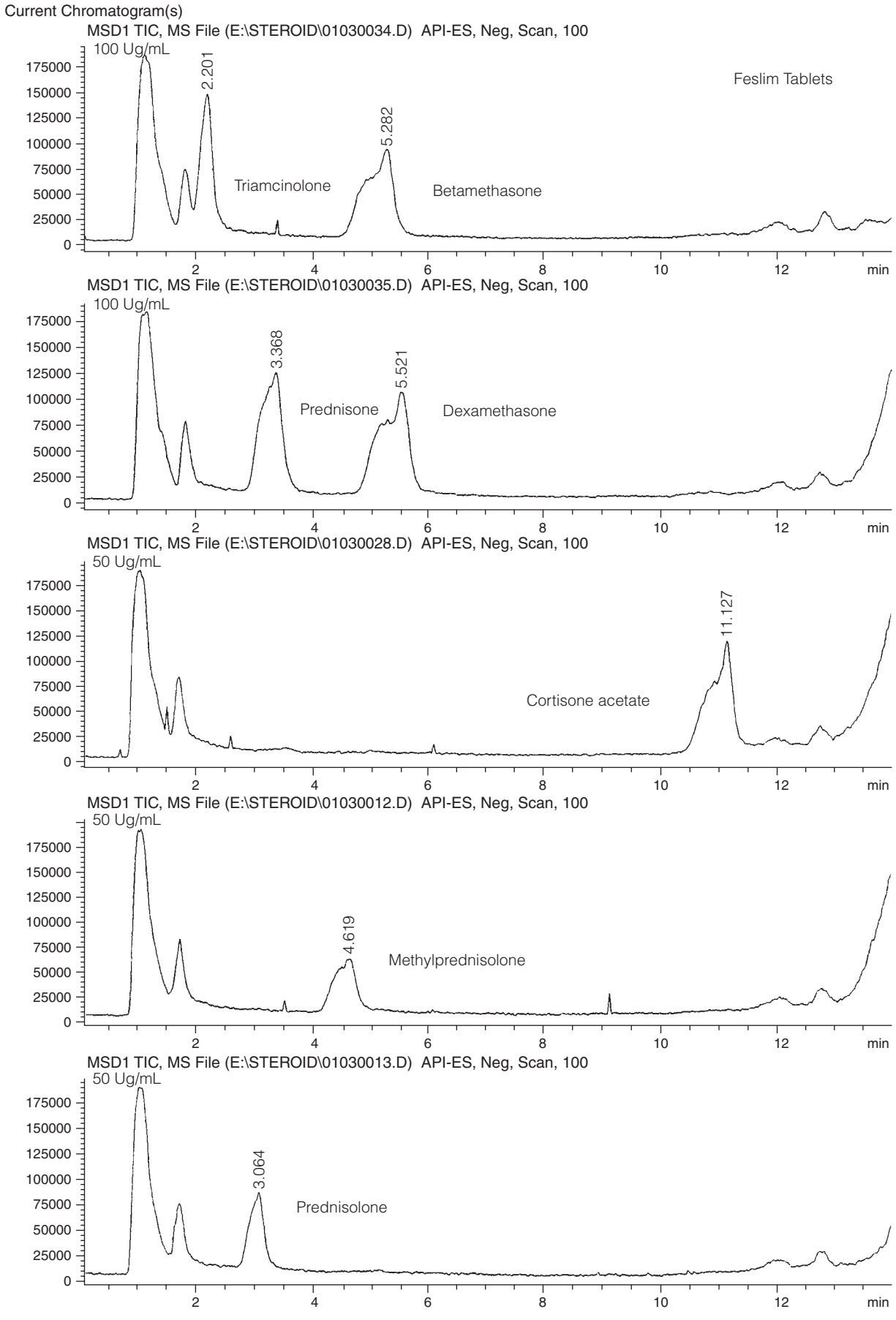

Figure 7. The LC/MS spectra of the spiked glucocorticoids in Feslim tablets.

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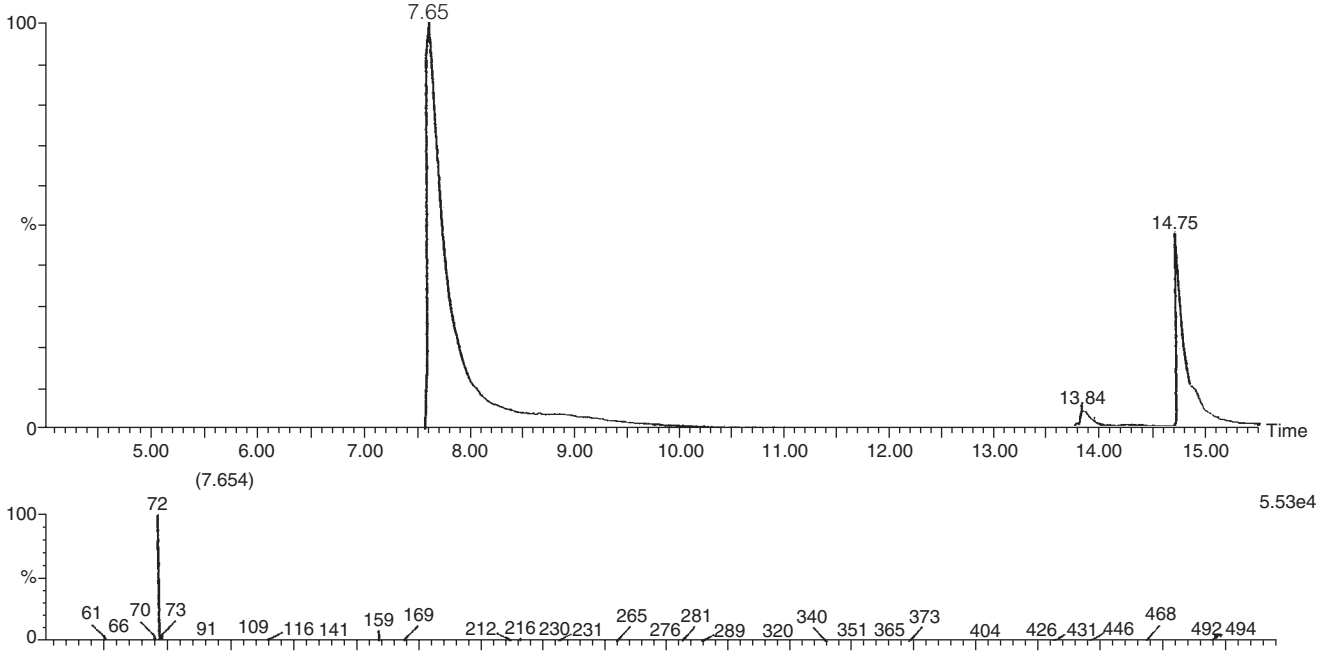

Figure 8. The GC/MS spectrum of fenfluramine of anorexics.

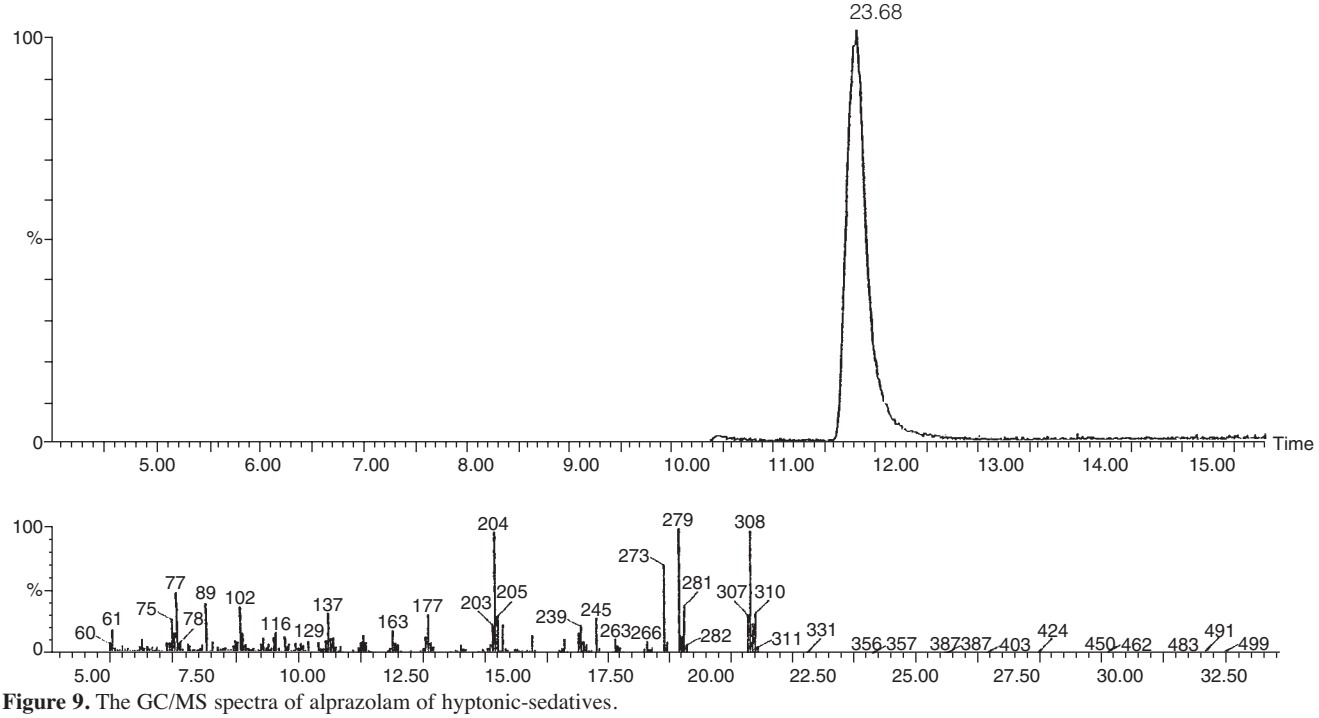

Figure 9. The GC/MS spectra of alprazolam of hyptonic-sedatives.

control standards and then identified with 3 different developing solvents to make sure the R_f values correlated with each another. The UV spectra of the drug components were established by analyzing the control standards with methanol as the spreading solvent. If we used different solvents, the spectra were slightly different. When analyzing the UV spectra, there were 3 drugs, barbital, phenobarbital and secobarbital sodium, that showed no maximum absorption wavelength. Those drugs without a maximum absorption wavelength, when developed in TLC, also showed weak absorption at UV 254 nm and the analyses should be enhanced with chromogenic analysis. The UV spectra of the spots in TLC, which dissolved in methanol, were used only as the control references. The identification of the optimal detection level was to evaluate the optimal concentrations of the 25 drug components of glucocorticoids. Generally, glucocorticoids are the drug components most frequently analyzed. To meet the analysis demand, the optimal detection

level of testing sample solution (1 μL) was analyzed with an LC/MS. The detection limit of glucocorticoids was 1.25 mg/g. The interferences with tailing peaks of the blank sample, Feslim tablets, in 7 different glucocorticoids are shown in Figure 4~7. The detection limit of the other drug categories was 0.1-0.4 mg/g, when 1 μL of testing sample solution was analyzed with GC/MS.

CONCLUSIONS

To accommodate the consideration of the Department of Health to protect consumers in food safety, not only efficiency, but also the accuracy in the determination of drug adulteration is also a demand. The study method we developed, separation of the drug constituents with a TLC method in combination with chromogenic reaction and a UV spectrophotometry, screening with an authentic standard drug, and then identification with other analytical instruments (e.g. GC/MS or LC/MS), can be a reference method for other researchers in this field. The study method established unified procedures and data, by reducing deviations from individual technicians, which makes it essential to implement standard operation procedures.

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