Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Pharmaceutical and Biomedical Analysis 41 (2006) 554–564
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Application of LC–ESI–MS–MS for detection of synthetic adulterants in herbal remedies

Maciej J. Bogusz*, Huda Hassan, Eid Al-Enazi, Zuhour Ibrahim, Mohammed Al-Tufail

Department of Pathology and Laboratory Medicide, King Faisal Specialist Hospital and Research Centre, 11211 Riyadh, P.O. Box 3354, MBC 10, Saudi Arabia

Received 15 October 2005; received in revised form 5 December 2005; accepted 7 December 2005
Available online 19 January 2006

Abstract

Adulteration of allegedly "natural herbal medicines" with undeclared synthetic drugs is a common and dangerous phenomenon of alternative medicine.

The purpose of the study was to develop a procedure for detection of most common synthetic adulterants in herbal remedies, using high-pressure liquid chromatography-electrospray tandem mass spectrometry (LC–ESI–MS–MS). Eighty drugs belonging to various pharmacological classes were included in the study. For most drugs two transitions were monitored, using protonated or deprotonated molecules as precursor ions. The drugs were isolated from herbal remedies using simple methanol extraction. Chromatographic separation was done in gradient of acetonitrile—10 mM ammonium formate buffer (pH 3.0). Drugs tested were grouped in suites, comprising analgesic drugs, antibiotics, antidiabetic drugs, antiepileptic drugs, aphrodisiacs, hormones and anabolic drugs, psychotropic drugs, and weight reducing compounds. These suites were used according to the declared benefits of examined preparations. Limits of detection ranged from 5 pg to 1 ng per injected sample. Drug-free herbal remedy spiked with eight various pharmaceuticals occurring in adulterated herbal preparations was used for internal proficiency testing. The recoveries of spiked drugs ranged from 63 to 100%. The procedure was applied in everyday casework. Several undeclared drugs were identified in "herbal" remedies, like e.g. sildenafil, tadalafil, testosterone, or glibenclamide. Pharmacological properties of detected drugs always corresponded with the claims of the "natural" remedies. The method presents a valuable extension of standard GC–MS screening used for this purpose.
© 2006 Elsevier B.V. All rights reserved.

Keywords: Herbal remedies; Synthetic adulterants; LC–MS–MS

1. Introduction

Ever-increasing use of herbal remedies of questionable quality exposes human population on multiple risks and creates a major concern for various health agencies on national and international level. World Health Organization (WHO) and European Union (EU) issued several guidelines and acts concerning safe and appropriate use of herbal medicines [1–3]. Safety issues related to herbal medicine are complex, and comprise possible toxicity of natural herbal constituents, presence of contaminants or adulterants, and potential interactions between herbs and prescription drugs. The quality of herbal medicines is often poor. The production of herbal remedies is not controlled or regulated. Persons involved in production, distribution and application of herbal remedies ("herbalists") very often do not have proper education and ethics [4,5]. Herbal medicines usually contain a range of pharmacologically active compounds. In some cases it is not known which of these constituents produces the therapeutic effect. Testing for efficacy in this situation is obviously more complex than with synthetic drugs [6,7]. Quality control systems based on chromatographic or electrophoretic fingerprinting were recommended evaluation of herbal remedies [8].

Adulteration of herbal remedies with undeclared synthetic drugs is a common problem, which may potentially cause serious adverse effects. Huang et al. [9] found that around 24% out of 2609 samples of traditional Chinese medicines analyzed in Taiwan were adulterated with synthetic drugs of various pharmacological activities. To most frequent drugs belonged: non-steroid anti-inflammatory drugs (NSAID), steroids, and analgesics. Koh et al. [10,11] reported similar adulteration profile in Chinese "herbal" remedies analyzed in Singapore. Ernst published a systematic review of 22 studies done on adulteration of Chinese herbal remedies with synthetic drugs in the period

* Corresponding author. Tel.: +966 1 442 4341, fax: +966 1 442 4280.
E-mail addresses: mbogusz@web.de, bogusz@kfshrc.edu.sa (M.J. Bogusz).

0731-7085/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2005.12.015

of 1990–2000. It was concluded that adulteration is a potentially serious problem, putting consumers at risk [12]. In several casuistic reports, the presence of codeine [13], dexamethasone and indomethacin [14] phenylbutazone [15,16], chlopropamide [17], or fenfluramine [18] was described in "herbal" remedies. In last years, herbal remedies used to enhance sexual activity were frequently adulterated with sildenafil or its analogues [19–21].

In our previous paper [22] we presented the results of the analysis of herbal remedies, tested in 2000–2001. Seventy-seven out of 247 samples were disqualified due to high heavy metal content, bacterial contamination or presence of toxic compounds on natural or synthetic origin. Synthetic adulterants were screened with gas chromatography-mass spectrometry (GC–MS). Other authors described screening procedures for herbal adulterants using GC–MS and high-pressure liquid chromatography with diode array detection (HPLC–DAD) [11,23]. However, several drugs exist which are far better detectable with LC–MS or LC–MS–MS. Gratz et al. applied LC–ESI–MS in full-scan mode for detection of sildenafil, tadalafil, and vardenafil in dietary supplements and herbal remedies [21]. Very recently, Liang et al. published a study on detection of nine most common adulterants in herbal remedies, using LC–ESI–MS–MS in MRM mode [24].

The purpose of this paper was to develop a procedure for detection of drugs, potentially used as herbal adulterants, using simple extraction procedure, HPLC separation and tandem mass spectrometric detection. To our best knowledge, such a procedure, comprising a comprehensive list of compounds, has been not published so far. Drugs selected for this study were chosen on the base of literature data and own experience as well.

2. Experimental

2.1. Material and reagents

Eighty drugs belonging to various pharmacological and chemical classes were selected for the study. Acetazolamide, amiloride HCl, amitriptyline HCl, androstendione, benzthiazide, betamethasone, bisacodyl, bumetanide, caffeine, cantharidin, carbamazepine, chlordiazepoxide HCl, chlorothiazide, chlorpheniramine maleate, chlorpropamide, chlortetracycline HCl, chlorthalidone, clenbuterol hydrochloride, clomipramine HCl, clopamide, codeine phosphate, cyclothiazide, dipyrone, demeclocycline HCl, desipramine HCl, dexamethasone, diazepam, diclofenac sodium, dihydrostreptomycin sesquisulfate, doxepin, ethacrynic acid, fenfluramine HCl, flumethasone, fluoxetine HCl, furosemide, glibenclamide, gliclazide, glimepiride, glipizide, hydrochlorothiazide, hydrocortisone, hydroflumethiazide, ibuprofen, imipramine HCl, indomethacin, ketoprofen, mefenamic acid, methylphenidate HCl, morphine HCl, norpseudoephedrine HCl, notriptyline HCl, oxytetracycline dihydrate, paracetamol, phenobarbital Na, phentermine HCl, phenylbutazone, phenytoin, prednisolone, prednisone, probenecid, salicylamide, salicylic acid, spironolactone, streptomycin sulfate, testosterone decanoate, testosterone isocaproate, testosterone phenylpropionate, testosterone propionate, theophylline, tolazamide, tolbutamide, triamterene, valproic acid Na, yohimbine HCl—were purchased from Sigma–Aldrich as pure (>95%) compounds. Oxyphenbutazone (99% purity) was supplied by LGC Promochem (UK). Sildenafil citrate (98% purity) was obtained from Jerusalem Pharmaceutical Co., Ltd.

From these compounds, stock solutions of 1 mg/ml in methanol or methanol–water were prepared. Following drugs were available only in pharmaceutical preparations: betamethasone valerate (Betnovate 0.1% solution from Glaxo, Saudi Arabia), tadalafil (Cialis 20 mg tablets from Spimaco-Eli Lilly and Co., Saudi Arabia), sibutramine HCl (Reductil 10 mg capsules from Abbott GmbH, Germany), pioglitazone HCl (Actos 30 mg tablets from Takeda Pharmaceuticals, USA). These drugs were extracted with methanol to the nominal concentration of 0.05 mg/l. For the LC–MS–MS experiments, final concentrations of drugs were 0.1–10 μg/ml in methanol–water (1:1).

Methanol and acetonitrile were of HPLC grade and supplied by Merck AG, Darmstadt, Germany.

2.2. Apparatus

LC–MS–MS analyses were performed using a TSQ Quantum LC/MS/MS, together with Surveyor AS Autosampler and quaternary LC Pump (Thermo Finnigan, San Jose, USA, provided by Dar Al-Zahrawi, Riyadh, Saudi Arabia). Electrospray ionization (ESI) source was used in positive and negative mode in the study.

2.3. Sample preparation

Herbal remedies were prepared in following way: the sample (several grams) was pulverized using a ZM 200 Ultracentrifugal Mill (Retsch GmbH, Haan, Germany) to obtain a homogenous and representative material. One gram of pulverized sample was extracted with 10 ml methanol for 30 min in round bottom test tube using laboratory rotator. The extract was centrifuged for 5 min at 3600 × *g*, 1 ml of supernatant was taken into Eppendorf tube and centrifuged again for 3 min at 16,000 × *g*. The supernatant was collected for LC–MS–MS examination.

Samples containing sugars (e.g. herbal honey) or liquid samples were extracted with 10 ml of dichlormethane–isopropanol (9:1). The extract was centrifuged for 5 min at 3.600 × *g*, 1 ml of supernatant was evaporated under nitrogen at 37 °C, reconstituted in 200 μl methanol in Eppendorf tube and centrifuged again for 3 min at 16.000 × *g*. The supernatant was collected for LC–MS–MS examination.

2.4. Preparation of quality assurance standard

Quality assurance of testing for herbal adulteration is a novel task. Certified herbal material containing synthetic adulterants of known identity and concentration is not available commercially. Similarly, no external proficiency program is offered on his field. For this reason, an in-house quality control material and procedure was developed, using commercially available herbal dietary supplement "Colon pure" from GNC, USA, Code 350623. This preparation, which contained mixed *Psyllium* seeds rusk, was at first checked for the absence of adulterants using GC–MS

Table 1
Applied gradient elution profile

Time (min)	Flow (μl/min)	*A* (%)	*B* (%)
0	300	95	5
5	300	95	5
30	300	20	80
40	300	20	80
45	300	5	95
70	300	5	95
70.1	300	95	5
75	300	95	5

A: 10 mM ammonium formate buffer; pH 3.0, *B*: acetonitrile.

screening procedure [22]. One hundred grams of herbal material was then homogenized with the ultracentrifugal mill and divided into 1 g portions in Eppendorf tubes. Each portion was then spiked individually with 20 μl of the mixture of reference drugs, containing codeine, fenfluramine, glibenclamide, tadalafil, phenylbutazone, prednisone, bisacodyl, and amitriptyline in concentration of 1 mg/ml each in methanol. The tubes were capped, vortexed for 30 s and stored at −20 °C until extraction. One gram of spiked herbal material was then extracted with 10 ml methanol as described above. The supernatant was collected for LC–MS–MS analysis. It should contain 50 ng of each drug per 25 μl injection volume at 100% recovery.

2.5. LC–MS–MS procedure

All compounds were at first analyzed individually in syringe infusion condition. Product ions and corresponding collision energies were stored in the method files. For most compounds, two transitions from protonated or deprotonated molecule were monitored. In the next step, all compounds were subjected to HPLC–ESI–MS–MS in MRM mode and the chromatographic data were collected.

The compounds were separated on Superspher 100 RP-18, 4 μm particle size, 125 mm × 3 mm column together with Superspher 100 RP-18 4 mm × 4 mm guard column (Merck AG, Darmstadt). The injection volume was 25 μl. Gradient elution was applied using 10 mM ammonium formate buffer, pH 3.0 (*A*) and acetonitrile (*B*) as shown on the Table 1.

In positive ESI, the voltage was set at 4.2 kV, sheath gas was set at 35, and auxiliary gas at 10 units. In negative ESI, the voltage was 2.7 kV, sheath gas 15, and auxiliary gas 5 units. The resolution (peak width) at first as third quadrupole was set at 0.7 u in both ionization modes. Scan time was set at 0.5 s.

2.6. Validation

The solutions of pure reference drugs were analyzed three times, on different days. Three injections were done at each day. From these experiments, mean retention time values and limits of detection (LOD) were calculated. As a LOD value, a signal/noise ratio higher than 10 was assumed.

Herbal quality assurance (QA) standard, prepared as in p. 2.4, was run with each experiment and each analyzed sample of suspected herbal remedy. Observed retention times, peak areas, and signal-to-noise ratios were stored and monitored.

Absolute recoveries of drugs added to QA standard were calculated through the comparison of the peak areas of extracted drugs with the peak areas for drugs added to the methanol extract of blank unspiked herbal material. These experiments were done in triplicate.

Possible influence of co-extracted matrix compound on detectability of target analytes was checked in following way: selected drugs were analyzed twice, once dissolved in pure methanol and second time added to the methanol extract of blank unspiked herbal QC material. The concentration of each drug was 1 ng/μl in the final solution, 10 μl were injected into LC–MS–MS. The area ratios: drug in methanolic extract/drug in methanol were calculated for each drug. These experiments were also done in triplicate.

Quantitative analysis of glibenclamide and sildenafil was done using external standardization procedure. Blank herbal QA material was spiked with glibenclamide and sildenafil to the concentrations of 0, 10, 100, 500, and 1000 ng/g. One gram samples of spiked material were extracted with 10 ml methanol. The extracts were centrifuged as in p. 2.3 and subjected to LC–MS–MS examination. The analysis was performed in MRM mode, using transitions and collision energies as listed in Table 2 for particular compound.

These experiments were done in duplicate, on three different days. All validation parameters (linearity, LOD, LOQ) were calculated using BEN 2.0 software [25] for the calculations the analytical limits according to the DIN 32645. According to this norm, the LOD corresponds to this concentration of the analyte, which is higher that the blank value in 50% of cases (beta-error = 50%) at the significance level of 99% (alpha-error = 1%). For LOQ, both alpha- and beta-errors are set at 1%.

Following formulas were used in this software:

$$X_{\mathrm{NG}} = \frac{S_y t_{f,\alpha}}{m}\sqrt{\frac{1}{N} + \frac{1}{\hat{N}} + \frac{\bar{x}^2}{Q_{xx}}}$$

$$X_{\mathrm{BG}} = k\frac{S_y t_{f,a}}{m}\sqrt{\frac{1}{N} + \frac{1}{\hat{N}} + \frac{(X_{\mathrm{BG}} - \bar{X})^2}{Q_{xx}}}$$

where $X_{\mathrm{NG}} = \mathrm{LOD}$, S_y is the residual standard deviation, t the t-value from the tables with $f = N-2$ and $P = 95\%$, m the slope, N the number of determinations in concentration level, $\hat{N}$ the number of levels, $\bar{X}$ the mean of working range, $Q_{xx} = \sum (X_i - \bar{X})^2$ and $X_{\mathrm{BG}} = \mathrm{LOQ}$.

3. Results and discussion

3.1. LC–MS–MS data

Tables 2 and 3 show chromatographic and mass spectrometric data for analyzed compounds, divided into positive and negative ions. Only in the case of five drugs: betamethasone, fluoxetine, phenobarbital, tadalafil, and valproic acid one product ion was monitored, due to very low intensity of the second product ion. In all other cases, two product ions were routinely monitored. This fulfills the recommendations of the European Union concern-

Table 2
Examined compounds (positive ions) arranged in alphabetical order

Compound	Parent ion (*m*/*z*)	Product ions (*m*/*z*)	CE	Rt (min)	LOD (ng)
Amiloride	230	171, 116	22, 38	14.10 ± 0.15	0.8
Amitriptyline	278	233, 202	28, 58	30.31 ± 1.7	0.02
Androstendione	303	285, 267	18, 20	23.44 ± 0.27	2.5
Betamethasone	393	241	10	21.89 ± 0.22	0.1
Betamethasone valerate	477	355, 279	12, 22	28.34 ± 0.25	0.05
Bisacodyl	362	184, 167	32, 50	27.60 ± 0.08	0.02
Caffeine	195	138, 123	22, 38	14.67 ± 0.12	0.1
Cantharidin	197	107, 95	10, 22	17.06 ± 0.10	1
Carbamazepine	237	192, 194	24, 38	22.40 ± 0.04	0.03
Chlordiazepoxide	300	282, 247	26, 38	21.70 ± 0.35	0.1
Chlorotetracycline	479	444, 154	24, 32	19.56 ± 0.20	0.5
Chlorpheniramine	275	230, 167	20, 44	26.77 ± 0.29	0.2
Chlorpropamide	277	175, 192	22, 16	23.64 ± 0.15	0.1
Clenbuterol	277	203, 132	24, 26	19.50 ± 0.08	0.15
Clomipramine	315	86, 58	22, 40	32.30 ± 1.04	0.002
Codeine	300	215, 165	28, 44	14.81 ± 0.47	0.02
Demeclocycline	465	448, 430	22, 26	18.27 ± 0.12	2
Desipramine	267	193, 72	42, 18	27.69 ± 0.86	0.02
Dexamethasone	393	373, 237	10, 22	21.87 ± 0.10	0.005
Diazepam	285	193, 154	28, 38	27.30 ± 0.06	0.05
Dihydrostreptomycin	584	263, 221	34, 50	02.12 ± 0.02	1
Doxepin	280	107, 165	30, 60	26.72 ± 0.21	1
Fenfluramine	232	159, 109	28, 46	23.66 ± 0.25	0.05
Flumethasone	411	253, 121	18, 36	22.11 ± 0.06	0.05
Fluoxetine	310	44	18	28.88 ± 0.57	0.2
Glibenclamide	494	369, 169	18, 38	28.67 ± 0.07	0.01
Gliclazide	324	127, 110	22, 38	26.85 ± 0.12	0.02
Glimepiride	491	352, 126	18, 38	29.23 ± 0.15	0.03
Glipizide	446	321, 286	16, 28	24.16 ± 0.09	0.01
Hydrocortisone	363	121,143	38, 50	20.18 ± 0.08	0.3
Imipramine	281	208, 193	30, 44	29.20 ± 0.73	0.05
Indomethacin	358	139, 111	26, 52	28.97 ± 0.41	1
Ketoprofen	255	209, 105	16, 28	25.23 ± 0.41	0.2
Methylphenidate	234	84, 56	22, 44	20.51 ± 0.99	0.01
Morphine	286	286, 165	10, 44	08.75 ± 0.51	0.005
Norpseudoephedrine	152	134, 117	10, 20	13.28 ± 0.28	0.04
Notriptyline	264	233, 202	18, 58	28.44 ± 0.44	0.02
Oxyphenbutazone	325	160, 162	24, 24	25.25 ± 0.05	0.05
Oxytetracycline	461	426, 443	22, 16	16.11 ± 0.26	0.8
Paracetamol	152	110, 65	20, 36	10.46 ± 0.32	0.03
Phentermine	150	133, 91	10, 24	17.87 ± 0.98	0.02
Phenylbutazone	309	188, 160	20, 32	30.19 ± 0.30	0.4
Phenytoin	253	182, 104	20, 38	22.42 ± 0.03	1
Pioglitazone	357	134, 119	43, 52	22.96 ± 0.25	0.05
Prednisolone	361	343, 147	10, 36	20.01 ± 0.05	0.05
Prednisone	359	341, 267	12, 20	20.25 ± 0.06	0.1
Sibutramine	280	139, 125	20, 38	31.80 ± 0.25	0.02
Sildenafil	475	283, 100	40, 32	23.70 ± 0.50	0.02
Spironolactone	341	141, 91	38, 50	27.30 ± 0.05	1
Streptomycin	582	263, 246	36, 38	02.16 ± 0.05	1
Tadalafil	390	268	18	23.67 ± 0.25	0.02
Testosterone decanoate	443	109, 97	38, 38	64.57 ± 0.92	0.1
Testosterone isocaproate	387	109, 97	40, 40	47.12 ± 0.52	0.1
Testosterone phenylpropionate	421	105, 97	40, 38	42.58 ± 0.65	0.1
Testosterone propionate	345	109, 97	26, 26	36.68 ± 0.70	0.1
Theophylline	181	124, 96	20, 24	12.89 ± 0.13	0.4
Tolazamide	312	115, 91	22, 38	25.49 ± 0.22	0.2
Tolbutamide	271	155, 91	20, 42	24.78 ± 0.26	0.6
Triamterene	254	237, 104	32, 44	17.06 ± 0.21	0.1
Yohimbine	355	212, 144	26, 38	20.61 ± 0.31	0.05

CE: collision energy for applied for particular transition, Rt: mean value ± standard deviation of nine measurements.

Table 3
Examined compounds (negative ions) arranged in alphabetical order

Compound	Parent	Products	CE	Rt (min)	LOD (ng)
Acetazolamide	221	83, 80	20, 28	12.72 ± 0.30	0.3
Benzthiazide	430	308, 228	26, 42	23.64	0.05
Bumetanide	363	319, 207	20, 24	25.83	1.5
Chlorothiazide	294	214, 179	34, 50	13.47 ± 0.05	1.2
Chlorthalidone	337	190, 146	22, 26	18.34	3
Clopamide	344	189, 167	34, 30	19.97	4.5
Cyclothiazide	388	322, 269	28, 32	24.30	0.5
Diclofenac	294	250, 214	12, 24	28.85 ± 0.02	0.1
Dipyrone	310	191, 175	18, 28	13.90 ± 0.21	0.5
Ethacrynic acid	301	243, 192	24, 95	24.95 ± 0.06	0.5
Furosemide	329	285, 204	18, 24	21.93 ± 0.17	0.01
Hydrochlorothiazide	296	269, 205	22, 26	14.34 ± 0.15	0.5
Hydroflumethazide	330	303, 239	24, 30	17.09	0.7
Ibuprofen	205	161	10	29.58 ± 0.12	0.5
Mefenamic acid	240	196, 192	22, 32	31.08 ± 0.13	0.03
Phenobarbital	231	188	10	19.72 ± 0.17	0.2
Probenecid	284	240, 140	22, 30	25.98 ± 0.12	0.01
Salicylamide	137	93, 65	20, 38	17.24 ± 0.24	0.5
Salicylic acid	136	118, 93	18, 24	17.06 ± 0.19	0.05
Valproic acid	143	143	2	25.88 ± 0.27	3

ing identification, since two MRM transitions from the ionized molecule of target compound give four points in the scale—a value regarded as sufficient for unequivocal identification [26].

The distribution of drugs over the elution time range shows that the majority of positive ionized compounds were eluted between 20 and 30 min. In the case of negative ionized drugs, about half of analyzed compounds eluted before 20 min. This elution profile is similar to our data obtained for over 300 drugs, analyzed in gradient of acetonitrile and triethylammonium phosphate buffer, pH 3.0, using diode array detection [27]. Despite chromatographic overlapping of numerous substances, possible cross-talk phenomenon was practically excluded. Cross-talk occurs when different analyzed compounds have the same product ion and elute at the same time. In the case of streptomycin and dihydrostreptomycin, the danger of misidentification is avoided since the second product is different. In the case of testosterone salts, they all give the same product ions, but elute at very different time.

Observed limits of detection show that all examined drugs are detectable at the level of nanograms per gram (ppt). Such

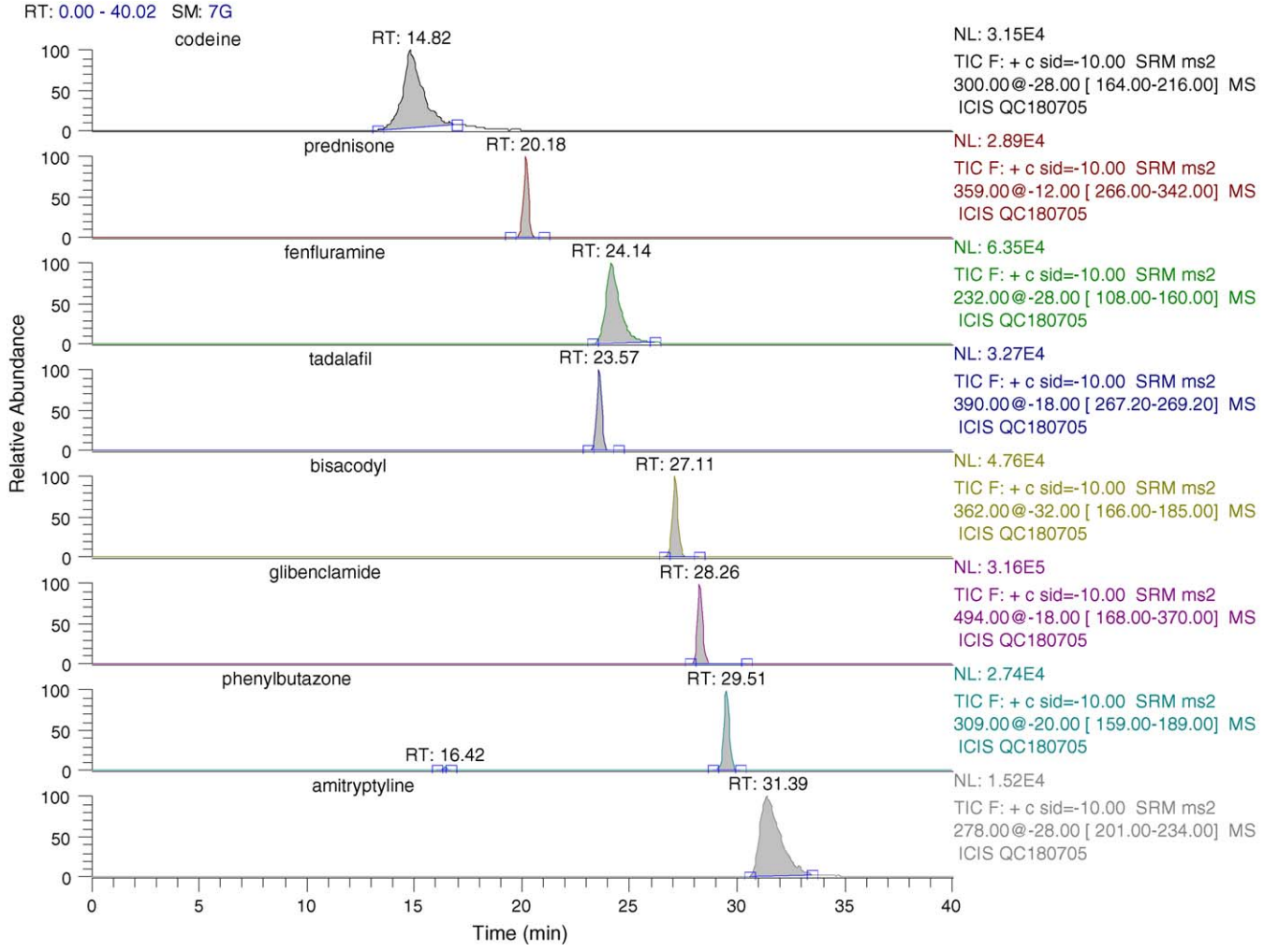

Fig. 1. Mass chromatogram of the extract of quality assurance herbal sample, spiked with eight drugs to the concentration of 20 mg/kg each. For each peak, a TIC (total ion chromatogram) of two transitions is shown.

Table 4
Analytical recovery as well as matrix effect of drugs analyzed in QA material

Compound	Recovery (%)	Matrix effect
Codeine	93 ± 3	0.87 ± 0.05
Prednisone	96 ± 4	0.91 ± 0.03
Fenfluramine	100 ± 5	0.87 ± 0.01
Tadalafil	92 ± 2	0.77 ± 0.04
Bisacodyl	95 ± 1	0.87 ± 0.02
Glibenclamide	94 ± 6	0.90 ± 0.05
Phenylbutazone	63 ± 5	0.61 ± 0.04
Amitriptyline	102 ± 4	0.97 ± 0.06

The recovery results are expressed as percentage of peak areas of pure, non-extracted compounds (mean ± R.S.D.). The matrix effects are expressed as ratio of peak areas of pure drugs added to extracted herbal blank material to peak areas of pure drugs dissolved in methanol.

sensitivity is more than sufficient for detection of adulterants in herbal remedies.

The examination of the blank herbal material, used as QA matrix, did not reveal any peaks corresponding to analyzed compounds. Fig. 1 shows typical mass chromatogram of the extract of our spiked QA standard—i.e. of the mixture of drugs, added to blank herbal material and extracted with methanol. The results of recovery studies as well as matrix effect are presented in Table 4. In seven out of eight drugs analyzed in QA mixture, the recovery was higher than 90%. Only in the case of phenylbutazone the recovery was around 60%. Coextracted matrix affected slightly the signal intensities for drugs tested; the peak areas of drugs dissolved in pure methanol were around 10% larger than the peak areas of drugs dissolved in blank herbal extract. For phenylbutazone, this effect was more pronounced.

Table 5
The results of validation of glibenclamide and sildenafil

	Linearity	R	S_y	LOD (μg/g)	LOQ (μg/g)	Precision[a]	Uncertainty[b] (%)
Glibenclamide	$y = 2549x + 258.4$	0.99926	245	0.36	1.25	8.1	24.3
Sildenafil	$y = 315x + 35.75$	0.99946	39.79	0.34	1.16	7.5	22.5

[a] Expressed as overall % R.S.D. at the levels of 0.1–1.0 μg/g.
[b] Expanded uncertainty at the level of confidence 99%.

Table 6
Examined compounds divided into suites

Suite	Compounds included
Analgesics—negative ions	Diclofenac, dipyrone, ibuprofen, mefenamic acid, salicylamide, salicylic acid
Analgesics—positive ions	Codeine, indomethacin, ketoprofen, morphine, oxyphenbutazone, paracetamol, phenylbutazone
Antibiotics	Chlorotetracycline, demeclocycline, dihydrostreptomycin, oxytetracycline, streptomycin
Antidiabetics	Chlorpropamide, glibenclamide, gliclazide, glimepiride, glipizide, pioglitazone, tolazamide, tolbutamide
Antiepileptics—negative ions	Phenobarbital, phenytoin, valproic acid
Antiepileptics—positive ions	Carbamazepine, phenytoin
Aphrodisiacs	Cantharidin, sildenafil, tadalafil, yohimbine
Hormones and anabolic drugs	Androstendione, betamethasone valerate, betamethasone, clenbuterol, dexamethasone, flumethasone, hydrocortisone, prednisolone, prednisone, testosterone propionate, testosterone isocaproate, testosterone phenylpropionate, testosterone decanoate
Psychotropic drugs	Amitriptyline, caffeine, chlordiazepoxide, clomipramine, desipramine, diazepam, doxepin, fluoxetine, imipramine, methylphenidate, norpseudoephedrine, notriptyline, theophylline
Weight reducers—negative ions	Acetazolamide, benzthiazide, bumetanide, chlorothiazide, chlorthalidone, clopamide, cyclothiazide, ethacrynic acid, furosemide, hydrochlorothiazide, hydroflumethiazide, metolazone, probenecid
Weight reducers—positive ions	Amiloride, bisacodyl, fenfluramine, phentermine, sibutramine, spironolactone, triamterene

Table 7
The results of positive findings in herbal remedies

Remedy	Claim	Analytical finding
Chinese herbal capsules "*Yong Gang*"	"Good health and stamina food supplement"	Tadalafil, sildenafil
Chinese herbal capsules "*Vigorous*"	"For natural general strength"	Sildenafil 49 mg/capsule
Herbal capsules "*Phyto Andro*"	"Tongkat Ali and other Asian herbs to nourish the body and fortify the male sexual function"	Sildenafil 18 mg/capsule
Instant herbal powder "*XKL*"	Drink "to enhance male strength"	Sildenafil 91 mg/g
Japanese fluid	"For women sexuality"	Testosterone decanoate
Unlabelled herbal tablets from Lebanon	Against diabetes. Recommended daily dose 15 tablets!	Glibenclamide 7.5 mg/tablet
Unlabelled herbal powder from Jordan	Against diabetes	Glibenclamide 4.5 mg/g
Unlabelled herbal capsules	"herbal slimming remedy"	Fenfluramine, phentermine, caffeine
Herbal powder *"Jamu Ragel"* from Indonesia	Against rheumatism and pain	Phenylbutazone, dipyrone

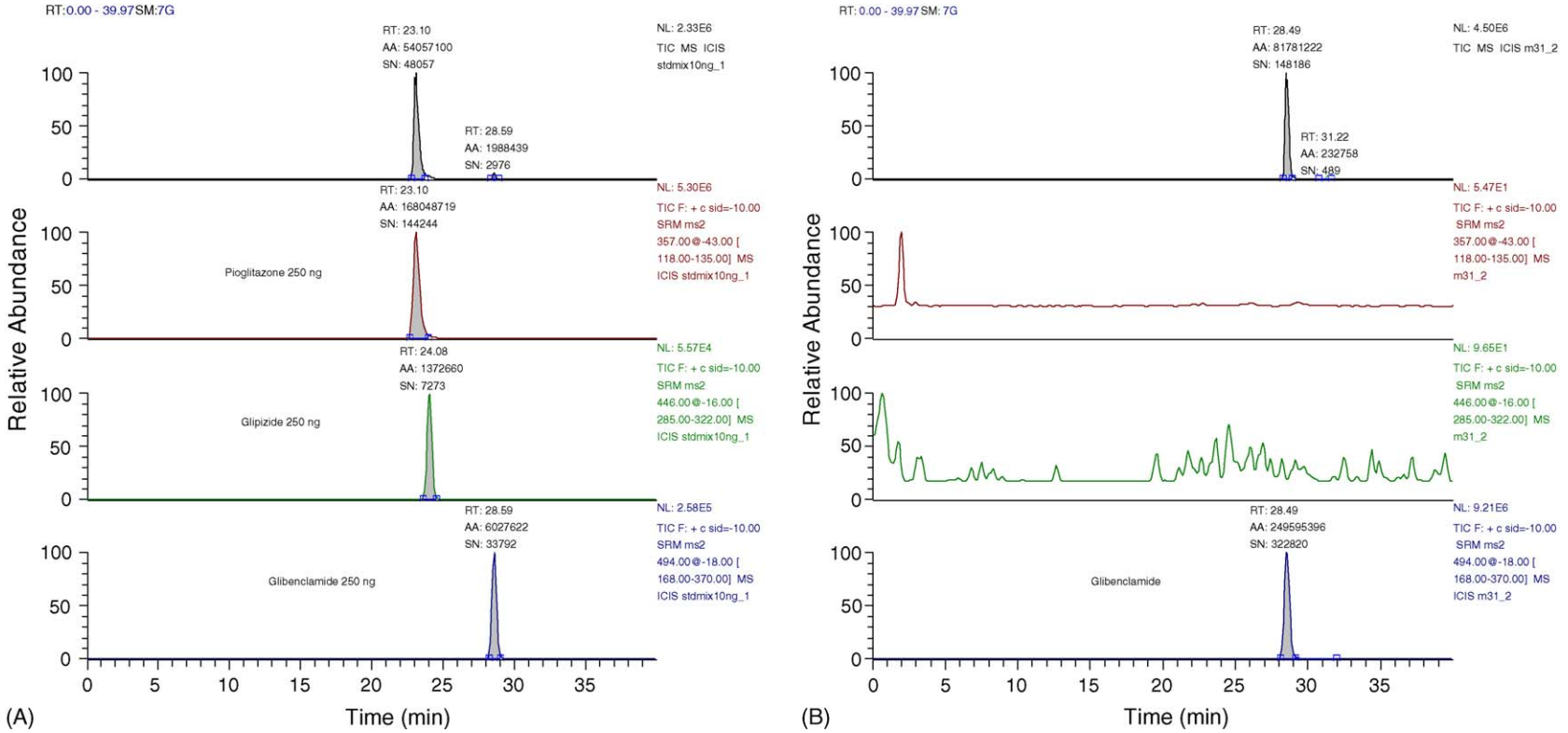

Fig. 2. Mass chromatogram of the mixture of selected antidiabetic drugs (A), mass chromatogram of the extract from “herbal” remedy against diabetes, containing glibenclamide. For each peak, a TIC (total ion chromatogram) of two transitions is shown.

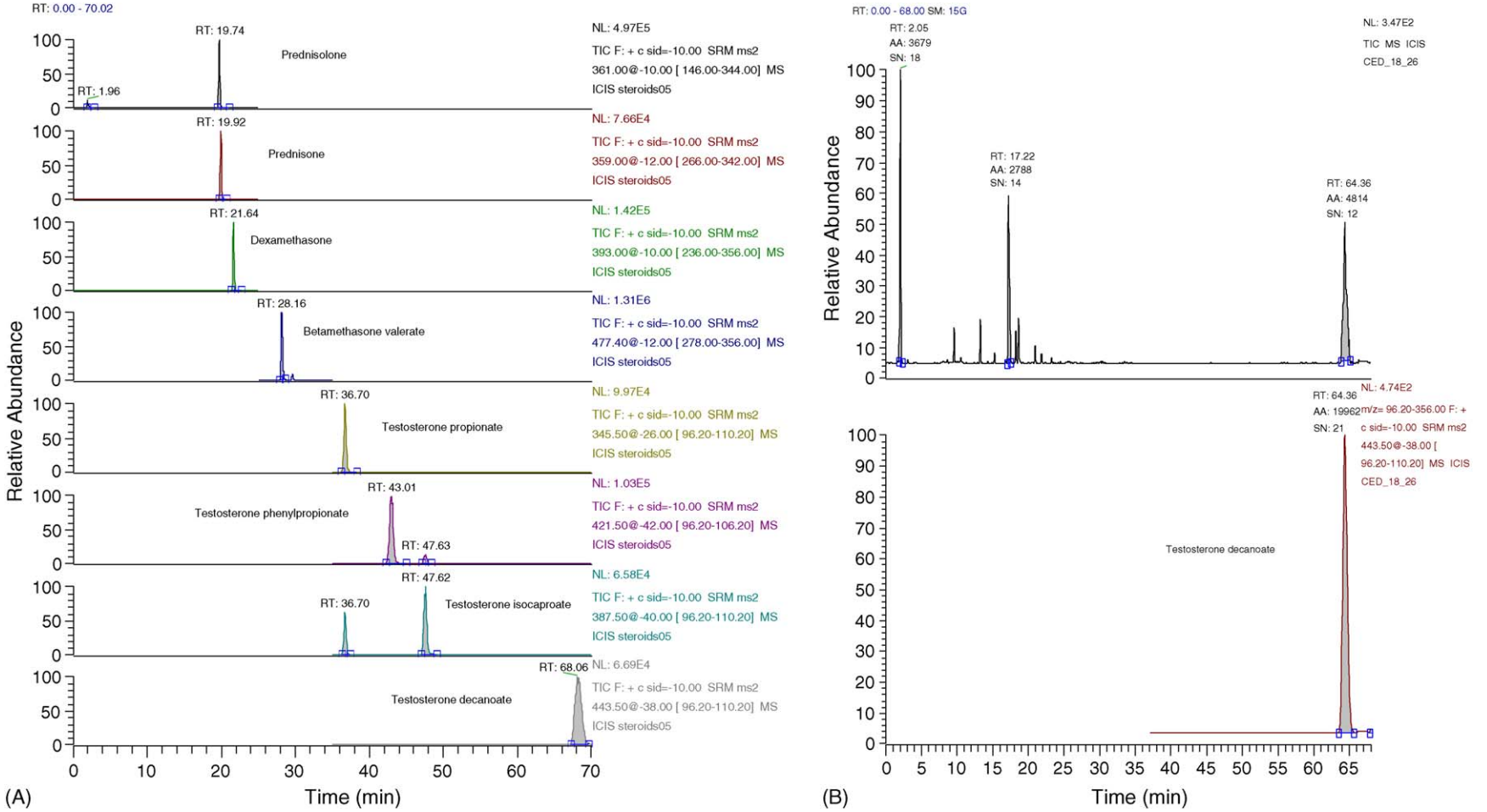

Fig. 3. Mass chromatogram of the mixture of selected steroids (A), mass chromatogram of the extract from Japanese remedy “for female sexuality”, containing testosterone decanoate (B). For each peak, a TIC (total ion chromatogram) of two transitions is shown.

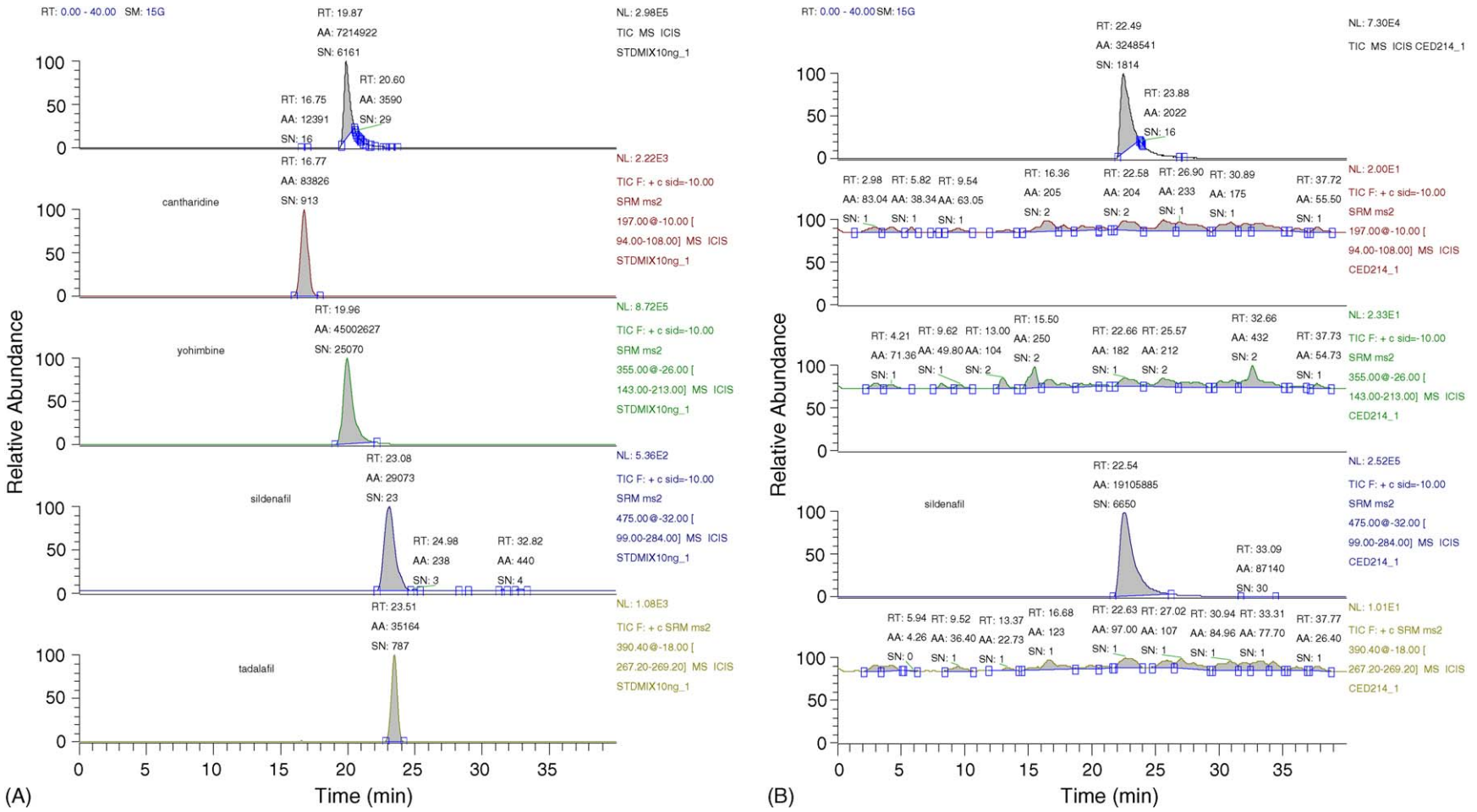

Fig. 4. Mass chromatogram of the mixture of aphrodisiac compounds (A), mass chromatogram of the extract from "herbal" capsules "*PhytoAndro*", containing sildenafil (B). For each peak, a TIC (total ion chromatogram) of two transitions is shown.

It must be stressed, however, that the herbal material used as QA matrix (*Psyllium* seeds rusk) represents the majority of herbal remedies, but certainly not all of them. Herbal preparations used as medicines are very variable in morphology and composition, and may appear as raw plants, plant parts (root, seeds, etc.), herbal capsules, herbal honey, herbal liquids or balms. Examination of QA material along with the samples is useful for monitoring the performance of the instrument and the method. The results, however, are representative only for pulverized, dried herbal preparations.

The validation data for the quantitative analysis of glibenclamide and sildenafil are shown in Table 5. The sensitivity of the method is very high in view of concentration of drugs found in herbal remedies. Therefore, in the case of positive results of qualitative screening for glibenclamide or sildenafil, it is advisable to dilute the primary methanolic extract 1:1000 with the methanolic solution of the appropriate internal standard.

The optimal application of collected LC–MS–MS data in everyday practice was subjected to scrutiny. It was tempting to develop a screening procedure for all examined drugs in one run. Such an approach was reported by Gergov et al. [28], who presented a LC–MS–MS screening procedure for 238 therapeutic drugs in blood. This attempt, however, has some limitations. For technical reasons, the library software permitted to include only one transition for each drug. Additionally, the monitoring of the large number of compounds involved is associated with very short scan times, what affects the sensitivity. Pilot study on time-scheduled monitoring, using five or more time windows, showed difficulties with the detection of compounds eluting close to the limits of each window.

For these reasons, the whole set of LC–MS data was divided into suites, comprising various groups of individual drugs. Applied scan time of 0.5 s allowed to obtain at least nine scans per peak for particular compound in suite. Appropriate suites were used according to the claim of a given product. Table 6 shows applied suites of drugs. The composition of these suites was dictated by practical observation, which showed that most of herbal remedies have only vague description or claim (“good for nerves”, “slimming herbal capsules”, “good for bones and joints”, or “for male strength”. Our previous experiences as well as the reports of other authors indicate that synthetic adulterants are usually added to achieve expected effect. Therefore, herbal remedies used, e.g. to lose weight may contain anorectics, laxatives, or diuretics, whereas drugs recommended to treat rheumatic disease may contain various analgesics or steroids. The division applied certainly does not follow the pharmacological rules; e.g. some laxative or diuretic drugs should not be regarded as weight reducers. However, these drugs have been applied as adulterants in various remedies to achieve temporary weight-reducing effect, at the expense of the health of the credulous user. Similar approach was applied by Gratz et al. [21] who analyzed selected synthetic phosphodiesterase inhibitors in herbal remedies using ESI–LC–MS. Liang et al. [24] took slightly different approach and screened for nine most common synthetic adulterants in herbal preparations with ESI–LC–MS–MS. Among these drugs were antibiotics, psychotropic drug, NSAID, phosphodiesterase inhibitor, and others. Italian authors [29] determined caffeine, theobromine, theophylline, and taurine in dietary supplements, using LC–ESI–MS in SIM mode.

3.2. Case examples

Described procedure has been applied for routine examination of various herbals remedies, available in Saudi Arabia. Following identification criteria were set: the signal/noise value of peak exceeding LOD, presence of all monitored product ions, and the retention time within ±2 S.D. values for particular compound. Various undeclared drugs were detected. In most cases, sildenafil or tadalafil were found in supposedly “pure herbal” remedies. Even more dangerous was detection of oral antidiabetic drugs in “herbal” capsules or tablets, used to cure diabetes. These preparations were provided with the instructions of use, which must unequivocally lead to severe hypoglycemia if followed. Table 7, as well as Figs. 2–4 show some examples from the casework. Applied approach, i.e. screening for particular groups of drugs according to the claims of the herbal product, appeared effective in the practical work. It confirms all previous observations indicating that herbal remedies are often deliberately laced with synthetic drugs to achieve desired action.

4. Conclusions

Developed LC–MS–MS screening procedure was successfully applied for determination of undeclared adulterants in herbal remedies.

LC–MS–MS is a valuable extension of toxicological screening of herbal remedies and enables detection of drugs, not amenable to GC–MS. Both techniques should be used for this purpose. The spectrum of drugs analyzed by LC–MS–MS should be permanently enhanced and completed.

References

[1] Guidelines for the Appropriate Use of Herbal Medicines, WHO Regional Publications, WHO Regional Office for The Western Pacific, Manila, 1998.
[2] WHO Guidelines on Safety Monitoring of Herbal Medicines in Pharmacovigilance Systems, WHO, Geneva, 2004.
[3] Mandate for the EMEA Working Party on Herbal Medicinal Products, www.emea.eu.int.
[4] H. Azaizeh, S. Fulder, K. Khalil, O. Said, Fitoterapia 74 (2003) 98–108.
[5] B.E. Abu-Irmaileh, F.U. Afifi, J. Ethnopharmacol. 89 (2003) 193–197.
[6] E. Ernst, M.H. Pittler, Complem Alt Med 86 (2002) 149–161.
[7] E. Ernst, in: M.N.G. Dukes:, J.K. Aronson (Eds.), Meyler’s Side Effects of Drugs, 14th ed., Elsevier Science, Amsterdam, 2000, pp. 1649–1681.
[8] Y.-Z. Liang, P. Xie, K. Chan, J. Chromatogr. B 812 (2004) 53–70.
[9] W.F. Huang, K.C. Wen, M.L. Hsiao, J. Clin. Pharmacol. 37 (1997) 344–350.
[10] H.L. Koh, S.O. Woo, Drug Saf. 23 (2000) 351–362.
[11] S.Y. Liu, S.O. Woo, H.L. Koh, J. Pharm. Biomed. Anal. 24 (2001) 983–992.
[12] E. Ernst, J. Int. Med. 252 (2002) 107–113.
[13] S.Y. Liu, S.O. Woo, M. Holmes, H.L. Koh, J. Pharm. Biomed. Anal. 22 (2000) 481–486.
[14] Health Sciences Authority Press Release, Singapore, 5 April 2004, www.hsa.gov.sg.

[15] Health Sciences Authority Press Release, Singapore, 23 January 2002, www.hsa.gov.sg.
[16] A.J. Lau, M.J. Holmes, S.O. Woo, J.H.L. Koh, J. Pharm. Biomed. Anal. 26 (2003) 401–406.
[17] D.M. Wood, S. Athwal, A. Panahloo, Diabet. Med. 21 (2004) 625–627.
[18] C. Corns, K. Metcalfe, Health J. R. Soc. 122 (2002) 213–219.
[19] L. Blok-Tip, B. Zomer, F. Bakker, K.D. Hartog, M. Mamzink, J. ten Hove, M. Vredenbregt, D. de Kaste, Food Addit. Contam. 21 (2004) 737–748.
[20] C. Shin, M. Hong, D. Kim, Y. Lim, Magn. Res. Chem. 42 (2004) 1060–1062.
[21] S.R. Gratz, C.L. Flurer, K.A. Wolnik, J. Pharm. Biomed. Anal. 36 (2004) 525–533.
[22] M. Bogusz, M. Al-Tufail, H. Hassan, Adv. Drug React. Toxicol. Rev. 21 (2002) 219–229.
[23] T. Snyman, M.J. Stewart, A. Grove, V. Steenkamp, Ther. Drug Monit. 27 (2005) 86–89.
[24] Q. Liang, J. Qu, G. Luo, Y. Wang, J. Pharm. Biomed. Anal. 2005, online at www.sciencedirect.com.
[25] M. Herbold, G. Schmitt, BEN Version 2. Programm zur statistischen Auswertung von Kalibrationsdaten nach DIN 32645, University of Heidelberg, Germany, 2000.
[26] EC – Commision Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, Off. J. Eur. Comm. L 221/8 of 7.8.2002.
[27] M.J. Bogusz, M. Erkens, J. Chromatogr. A 674 (1994) 97–126.
[28] M. Gergov, I. Ojanpera, E. Vuori, J. Chromatogr. B 795 (2003) 41–53.
[29] E. Marchei, M. Pellegrini, R. Pacifici, I. Palmi, S. Pichini, J. Pharm. Biomed. Anal. 37 (2005) 499–507.