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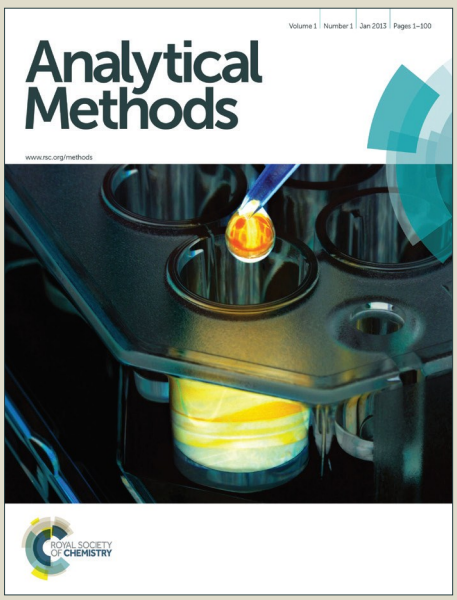

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Simultaneous analysis of antihypertensive and diuretics as adulterants in herbal-based products by ultra-high performance liquid chromatography-electrospray tandem mass spectrometry

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The world consumption of herbal-based products has increased substantially for the treatment, prevention and cure of certain diseases. However, the ineffective control of the available products has been contributing to marketing of products sold as "natural", which often contain illegally synthetic drugs as declared or non-declared components. It has been a common practice especially regarding drugs for treating chronic diseases, such as hypertension. In order to investigate herbal-based formulations with the claim of hypotensive activity, we developed an analytical method using ultra-high performance liquid chromatography-electrospray tandem mass spectrometry (UHPLC-ESI-MS/MS) for simultaneous determination of 13 antihypertensive drugs including diuretics, β-blockers, angiotensin II receptor antagonist and angiotensin converting enzyme inhibitors. Separation was accomplished in 6 minutes using a Zorbax SB-C_{18} column using methanol and acetic acid 0.1 % as mobile phase. Limits of detection ranged from 0.02 to 2.51 μg L^{-1} and accuracy from 80.56 to 111.28 %. A simple extraction procedure was used in the pretreatment step by dissolving the samples in methanol 100 % followed of a 1,000-fold dilution in the mobile-phase and filtration through a Teflon membrane (0.2 μm). No adulterants were detected in the formulations as non-declared drugs. However, five samples contained the diuretics hydrochlorothiazide and furosemide as declared on the label. Quantification of diuretics in these samples reveled doses above and below the recommended dose for furosemide and hydrochlorothiazide.

Keywords: Adulteration; antihypertensive; diuretics; herbal products; UHPLC-ESI-MS/MS.

Introduction

The world consumption of herbal-based products has increased substantially, since they aim to contribute for the treatment, prevention and cure of certain diseases. Because of their natural origin, this kind of formulation may be perceived as safe and less expensive alternatives to the use of conventional prescription drugs.[1] However, the lack of an effective control of all products available on the market contributes to marketing of herbal products of questionable quality. Furthermore, the current regulatory scenario worldwide contributes to the unethical practice of manufacturers to place medications and other active pharmaceutical ingredients in formulations marketed as "natural" to boost sales.[2-4] In this context, drugs widespread used for treating chronic illnesses such as hypertension, diabetes mellitus, arthritis, or for conditions such as obesity/overweight or erectile dysfunction are frequently found as adulterants in herbal-based products.[5-10]

Antihypertensive drugs (β-blockers, angiotensin converting enzyme inhibitors, angiotensin II receptor antagonist, diuretics) as adulterants have been reported and may be present in herbal products with hypotensive activity.[11] According to the World Health Organization (WHO), one in three adults worldwide has raised blood pressure, a condition that causes around half of all deaths caused by stroke and heart diseases.[12] Thus, arterial hypertension is a chronic disease, which must be constantly monitored and generally requires continued treatment with antihypertensive drugs in association or not. Furthermore, patients seek for alternative treatment with natural products has been observed as a common practice even when treated with conventional medicines. Whether these natural products are adulterated with antihypertensive drugs, this patient could intake excessive amount of antihypertensive drugs and develop a severe case of hypotension and bradycardia.[13]

Since the adulteration of natural products with undeclared synthetic drugs is a serious problem which puts in risk consumers' health, there is a concern to reveal these frauds through the development and application of analytical methodologies to investigate the occurrence of adulteration. In this context, Liang et al.[14] developed a method using liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the determination of the nine most common adulterants in herbal products and food

supplements found in Chinese market, among them were two antihypertensive drugs, captopril and nifedipine. Another analytical methodology published in the same year involved the determination by LC-MS/MS of drugs from different pharmacological classes, in which 14 diuretics were studied as weight reducer adulterants.[15] Seven blood pressure agents (indapamide, reserpine, nicardipine, captopril, lisinopril, methyldopa, and elanaprilat) were also studied by Chen et al.[11] in the analysis of adulterants in 105 dietary supplements. In this study, thirty-five samples were adulterated, but none with the antihypertensive drugs.

Regarding the analytical approaches for antihypertensive and diuretics, an analytical method using LC/MS was developed for 18 antihypertensive drugs, including diuretics, calcium antagonists, and angiogenesis-converting enzyme inhibitors (ACEI) as adulterants. The method was applied in the analysis of 35 samples and detected the presence of hydrochlorothiazide as well as association of hydrochlorothiazide with clonidine or triamterene.[10] In another work, a simultaneous analysis of 17 diuretics in dietary supplements by HPLC and LC-MS/MS was performed by Woo et al.[16] In China, Gold Nine Soft Capsules, an "herbal-based" medicine intended for treatment of hypertension, was investigated and three anti-hypertensive drugs (amlodipine, indapamide, and valsartan) were identified by LC-HRMS and NMR.[17]

The herbal-based formulations studied in this work and marketed by compounding pharmacies are not classified as phytotherapeutic medicines according to the current Brazilian legislation. The current regulation on phytomedicines in Brazil is the RDC 26/2014 of National Health Surveillance Agency (ANVISA), which regulates the registration of "Herbal Medicines" as well as the registration and notification of "Traditional Phytotherapeutic Products".[18] These products are manufactured strictly containing herbs and on an industrial scale. Concerning the regulatory aspects on compounding pharmacies (magistral scale), the resolution 67/2007 of ANVISA establishes the Good Practices of Manipulation of formulations for human use.[19] These compounding formulations containing plants are considered extemporaneous and under prescription, thus should be prepared for each patient.

Considering that the presence of undeclared synthetic substances is not allowed in herbal products or dietary supplements and the practice of adulteration has been recurrent worldwide, the purpose of this paper was to develop an analytical method for simultaneous detection and quantification of 13 antihypertensive drugs,

including some diuretics in herbal-based products by UHPLC-ESI-MS/MS. In relation to the existent methods and works dealing with the analysis of these drug classes, the present work bring together the most probable antihypertensives and diuretics studied by analytical methods in a single run. Furthermore, the study of the 13 selected drugs in formulations marketed as herbal-based products by compounding pharmacies was not reported up to date. The drugs were chosen because they are the most commonly prescribed by physicians for the treatment of hypertension in Brazil. The method was applied to the screening and quantification of the drugs in 34 formulations marketed as alternative treatment for high blood pressure in different Brazilian regions.

Experimental

Standards and reagents

Propranolol hydrochloride, atenolol, metoprolol tartrate, and nadolol (β-blockers), captopril and enalapril maleate (angiotensin converting enzyme inhibitors), losartan and valsartan (angiotensin II receptor antagonist), furosemide, hydrochlorothiazide, chlorthalidone, amiloride, and spironolactone (diuretics) were all of pharmaceutical grade, as verified by a certificate of analysis. Methanol and acetonitrile LC-MS grade were obtained from Panreac (Barcelona, Spain) and Fluka (St. Gallen, Switzerland) respectively. Acetic acid 99.7 % and formic acid 95 % were obtained from Sigma-Aldrich (St. Louis, MO).

Instrumentation and apparatus

Antihypertensive and diuretic drugs were determined by using an UHPLC-ESI-MS/MS system from Agilent Technologies (Santa Clara, CA, USA) with a chromatograph model 1260 Infinity consisted of a binary pump with automatic injector and a mass detector 6430 triple quadrupole. The separation was performed with a Zorbax® SB-C_{18} column (Agilent) (2.1 x 50mm, 1.8μm). Electrospray ionization (ESI) source was used for being more suitable for neutral or polar

substances that may be protonated and deprotonated in appropriate conditions of pH. ESI source was used in positive and negative mode in the study.

Samples of herbal-based products

We searched the Internet for compounding pharmacies advertising herbal products in 8 different Brazilian states (i.e., Ceará, Distrito Federal, Goiás, Minas Gerais, Paraná, Rio Grande do Sul, Santa Catarina, and São Paulo). Based on this Internet search, two research assistants contacted compounding pharmacies by e-mail, telephone or in person to request any available herbal product for treating high blood pressure. We received via express mail 34 herbal preparations from 30 pharmacies. Although natural products had been requested as alternative treatment for high blood pressure, some samples contained synthetic drugs that were declared on the package label, including the diuretics furosemide and hydrochlorothiazide. The samples had active ingredients and composition as described by the manufacturers and listed in Table 1. The samples were stored at room temperature and used as received for analysis. We documented the labeled components of each product and then analyzed for the studied antihypertensive and diuretic drugs using the described methodology.

TABLE 1

Analytical Procedure

Stock standard solutions (1000 mg L^{-1}) were prepared by weighing and dissolving each analyte in methanol. Stock solutions were stored at 4 ^{o}C. Standard working solutions were prepared by mixing and appropriate dilution of stock solution in methanol. All solutions were filtered in Teflon filter (0.2 μm) before injection in the UHPLC system. For determination of adulterants in the samples (capsule or tablet) the average weight of 20 capsules/tablet was obtained and a sample pools was prepared. The equivalent weight of 1 capsule was then dissolved in 25 mL of methanol in a volumetric flask. The solution was then diluted 1,000-fold before filtration through a Teflon filter (0.2 μm) and injection in the UHPLC system for the screening of the sample. Once detected the analyte in the screening step, the sample

was properly diluted for quantification. The quantification of adulterants in the samples was performed by using the standard addition method (n = 3).

The UHPLC-MS/MS separation of 13 drugs was carried out using a mobile phase composed of methanol/0.1 % acetic acid in a gradient elution program consisting of 15 % methanol/0.1 % acetic acid in the first 0.5 min, 55 % methanol/0.1 % acetic acid up to 3.0 min, and 100 % methanol until the end of the chromatographic run (6.0 min). An equilibration time of 4.0 min was used to return to the initial mobile-phase composition and equilibrate the gradient before the next sample injection. During all the chromatographic run, the column was maintained at 50 ^{o}C. The flow rate was 0.6 mL min^{-1} and injection volume was 2 µL.

Results and discussion

Optimization of MS conditions

The MS detector worked with an electrospray ionization source (ESI) as an interface for ionization process. The MS parameters were optimized by injection of each adulterant directly in the mass spectrometer. The condition chosen was one that offered highest signal intensity in the spectrum obtained for the studied adulterants. The optimized source parameters were gas temperature, gas flow, nebulizer pressure and capillary voltage. The gas temperature was studied in a range from 200 to 350 ^{o}C, where lower temperatures lead to the weak ionization of drugs by decreasing the ability to dry the mobile phase. Gas flow was also studied for the same purpose in a range from 6 to 12 L min^{-1}. Nebulizer pressure was analyzed in the range from 10 to 50 psi in order to assess the best pressure for the formation of the droplets in the electrospray. And finally the capillary voltage was optimized in a range from 1000 to 4000 V to evaluate the efficiency for charging the droplets. An increase of sensitivity was obtained for all adulterants at 350 ^{o}C of gas temperature, 10 L min^{-1} of gas flow, 30 psi of nebulizer pressure and 2000 V of capillary voltage.

In order to analyze the drugs by multiple reaction monitoring (MRM), the fragmentation and abundances of the product ions for the quantification were studied by optimizing the parameters of fragmentors (50–300 V), collision energies (0–30

eV) and accelerator cell voltage (0–8 V). Spironolacton was the only adulterant analyzed by the specific ion monitoring (SIM), because the signal intensity of its product ion was very low. The retention time, precursor ion, product ion, fragmentor, collision energy and cell accelerator voltage for each analyte are shown in Table 2.

TABLE 2

Determination of anti-hypertensive and diuretics by UHPLC-ESI-MS/MS

To perform the separation and detection of anti-hypertensive and diuretics as adulterants, different mobile phase modifiers (methanol and acetonitrile) and additives such as ammonium acetate, ammonium formate (5, 10, 20, and 50 mM), acetic acid, formic acid, and ammonium hydroxide (0.05, 0.1, 0.5, and 1.0 %) were systematically studied. All additives were able to ionize the adulterants, but in general, higher concentrations of additive led to low signal intensity. Thus, 0.1 % (v/v) acetic acid was chosen because it provided higher signal intensity for most studied adulterants. Furthermore, methanol was chosen as organic solvent because it allowed the separation of adulterants in a shorter chromatographic run comparing to acetonitrile. In addition, the use of acetonitrile resulted in a fronting deformation of the chromatographic peak for captopril. Therefore, the separation of 13 adulterants was accomplished using a gradient elution program, which consisted of 15 % methanol/0.1 % acetic acid in the first 0.5 min, 55 % methanol/0.1 % acetic acid up to 3.0 min, and 100 % methanol until the end of the chromatographic run after 6.0 min. In addition, a time of 4.0 min was necessary to return to the initial mobile-phase composition and equilibrate the gradient before the next sample injection. During all the chromatographic run, the column was maintained at 50 ^{o}C. The flow rate was 0.6 mL min^{-1} and injection volume was 2 μL. In the chromatogram shown in Figure 1, it is possible to observe that some adulterants were not completely separated if visualized in the total ion chromatogram (TIC). However, when precursor and product ions are extracted from TIC and analyzed by MRM mode, it is possible to distinguish the signal of each analyte, so the co-elution problem can be solved (Figure 2).

FIGURE 1

FIGURE 2

Method validation by UHPLC-ESI-MS/MS

After optimization, the method was validated considering the major analytical validation parameters for the studied adulterants. The method validation was based in the Brazilian validation guide for analytical and bioanalytical methods (RE nº 899, 2003)[20]. The linear range was obtained by injecting in triplicate each concentration level (at least seven levels) varied from 1.95 to 500 μg L^{-1}. The obtained correlation coefficients were all higher than 0.99. The detection and quantification limits were calculated from 3σ and 10σ values, respectively; the standard deviation was obtained by seven measurements of the background noise. The precision was expressed by the variation coefficients (expressed as RSD) of the results obtained in triplicate for all levels of the linear range for each analyte. For the accuracy calculation, the standard addition method (n = 3) was used, where three different concentrations of the standard solution were added into a sample that already contained a known concentration of each adulterant prior to the extraction and filtration process. The results obtained for the linear range, LOD, LOQ, precision, and accuracy are shown in Table 3. As can be seen, precision ranging from 0.03 to 3.93 % and accuracy ranging from 80.56 to 111.28 % are also in agreement with the AOAC requirements for validation results experiments in botanicals and dietary supplements, mainly considering the studied concentration levels.[21]

TABLE 3

Analysis of commercial formulations of herbal-based products

The proposed method was applied in the analysis of 34 samples of herbal-based products acquired from compounding pharmacies in the Brazilian market. Although all samples were sold as natural product, five samples (7, 9, 16, 17, and 21) contained synthetic diuretics as declared on the formulation label. In all other samples, there were not detected any of the studied adulterants. Five samples that

contained diuretics furosemide or hydrochlorothiazide as a declared drug were then quantitatively studied by the proposed UHPLC-ESI-MS/MS. Firstly the samples had to be diluted in methanol several thousand folds, so that the adulterant could be quantified within the linear range of the validated method (Table 3). Sample dilution was also useful to minimize the matrix effect. Furthermore, the standard addition method was used for quantification, which is always considered a fit-for-purpose approach for elimination of the matrix effect on analyte signals. Of the samples analyzed, only sample 7 contained hydrochlorothiazide as a declared dose on the label (10 mg/capsule). None of the other formulations contained the declared dose of diuretic. Table 4 lists the recommended daily doses for hydrochlorothiazide and furosemide and the real ingested doses after quantification by UHPLC-ESI-MS/MS and following the recommendation for consumers on the label.

TABLE 4

Considering the concentrations found in the formulations and the recommended daily doses for hydrochlorothiazide (12.5–25 mg/day) and furosemide (20–40 mg/day), samples 16 and 17 contained approximately twice the recommended dose for hydrochlorothiazide (39.98 mg) and furosemide (92.55 mg). Figure 3 shows the chromatograms obtained from the analysis of two samples containing diuretics in the formulation. Samples 9 and 21 contained furosemide and hydrochlorothiazide, respectively, and the respective chromatograms of the extracted ion confirmed the presence of both diuretics in the samples.

FIGURE 3

Conclusion

Adulteration of natural products with synthetic drugs is a recurrent problem worldwide, especially associated with the poor quality control over the production and marketing of these formulations. The investigation of adulteration cases requires analytical methods of high specificity due to the complexity of the samples. Thus, the use of separation techniques, such as liquid chromatography coupled to mass

detector, has been a confirmatory analytical tool to identify and quantify adulterants in these formulations. In this work, an analytical method was developed by using UHPLC-ESI-MS/MS for the determination of 13 antihypertensive drugs in herbal products. The method was applied in the analysis of 34 samples of natural products commercialized in the Brazilian market for the alternative treatment of hypertension. None of the samples was adulterated with the drugs as undeclared components, although five of them contained the declared presence of hydrochlorothiazide or furosemide. These drugs were quantified and, according with the suggested daily intake, one sample presented under dosing of hydrochlorothiazide. Furthermore, two samples contained the dose within the recommended daily dose values and two samples presented overdoses of nearly twice the recommended dose for hydrochlorothiazide and furosemide. Anyway, considering that antihypertensive and diuretic drugs are not classified as over-the-counter drugs, the intentional addition (declared or not) to herbal-based products violates the current rules all over the world. Once the dose is not declared and the formulation is marketed as a natural product, the possibility that a patient has been making use of these products simultaneously with other medicines is high, considering arterial hypertension is a chronic disease that requires continuous treatment and monitoring of arterial pressure. The combination of these drugs resulting in an overdosage can lead to the development of severe hypotension, beyond the risk of adverse drug interaction due to a synergistic effect among drugs.

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Captions to the figures

Figure 1: Total ion chromatogram (TIC) of 13 antihypertensive and diuretic adulterants (1) atenolol (1 mg L^{-1}), (2) amiloride (1.0 mg L^{-1}), (3) hydrochlorothiazide (5.0 mg L^{-1}), (4) nadolol (1.0 mg L^{-1}), (5) metoprolol tartrate (1.0 mg L^{-1}), (6)captopril (2.0 mg L^{-1}), (7) chlorthalidone (5.0 mg L^{-1}), (8) furosemide (5.0 mg L^{-1}), (9) propranolol hydrochloride (1.0 mg L^{-1}), (10) enalapril maleate (1.0 mg L^{-1}), (11) losartan (0.5 mg L^{-1}), (12) spironolactone (0.625 mg L^{-1}), (13) valsartan (1.25 mg L^{-1}). Gradient elution: 15% methanol/0.1% acetic acid (0–0.5 min); 55% methanol/0.1% acetic acid (0.5–3.0 min); 100% methanol (3.0–6.0 min). Source parameters: 350 ^{o}C of gas temperature, 10 L/min of gas flow, 30 psi of nebulizer pressure, and 2000 V of capillary voltage.

Figure 2: Extracted chromatogram (MRM) of 13 antihypertensive and diuretic as adulterants: (1) atenolol, (2) amiloride, (3) hydrochlorothiazide, (4) nadolol, (5) metoprolol tartrate, (6) captopril, (7) chlorthalidone, (8) furosemide, (9) propranolol hydrochloride, (10) enalapril maleate, (11) losartan, (12) spironolactone, (13) valsartan. Other conditions as described in Figure 1.

Figure 3: Chromatogram obtained from the analyses of samples 9 and 21 (Table 1). (a) Total ion chromatogram (TIC) with peaks detected in the retention time of furosemide (sample 9) and hydrochlorothiazide (sample 21); (b) Chromatogram extracted for transitions of furosemide (329.1>285.2) in sample 9 and hydrochlorothiazide (296.0>269.0) in sample 21. Other conditions as described in Figure 1.

Table 1. Herbal products acquired from compounding pharmacies marketed as alternative treatment for high blood pressure

Sample	Composition declared on the formulation label	Average weights tablet/capsule (g)
1	*Camelia sinensis* (green tea) 450 mg	0.4856
2	*Garcinia cambogia* 200 mg, *Equisentum* sp. 500 mg, *Cassia augustifolia* 50 mg and *Phytolacca decandra* L. 150 mg	0.5290
3	*Rhamnus purshiana*, *Centella asiatica*, *Cynara scolymus*, *Baccharis trimera*, *Fucus vesiculosus*, *Equisentum* sp., *Cassia augustifolia, Spirulina maxima* and *Passiflora* sp.	0.3845
4	*Rhamnus purshiana*, *Camelia sinensis* (green tea), *Fucus vesiculosu*s and *Garcinia cambogia*	0.7193
5	*Equisentum* sp., *Cassia augustifolia*, *Centella asiatica*, *Cynara scolymus*, *Fucus vesiculosus* and *Amorphophallus konjac*	0.3452
6	*Cordia ecalyculata* Vell, Slendesta™, *Camelia sinensis* (green tea)	0.5576
7	*Fucus vesiculosus*, *Centella asiatica*, *Spirulina máxima*, *Passiflora* sp., *Rhamnus purshiana*, caffeine and hydrochlorothiazide	0.4438
8	*Cynara scolymus*, *Rhamnus purshiana*, *Equisentum* sp., *Fucus vesiculosus* and *Ptychopetalum olacoides* B.	0.5351
9	*Cassia augustifolia*, *Fucus vesiculosus*, furosemide, *Rhamnus purshiana*, *Garcinia cambogia* and *Cyamopsis* sp.	0.5497
10	*Cynara scolymus*, *Fucus vesiculosus*, *Spirulina maxima*, *Rhamnus purshiana* and *Centella asiatica*	0.4205
11	*Cordia ecalyculata* Vell	0.2984
12	*Garcinia cambogia*, *Rhamnus purshiana*, *Fucus vesiculosus*, *Cynara scolymus*, *Equisentum* sp.,	0.4295

	Amorphophallus konjac, *Cassia angustifolia*, *Centella asiatica*, *Ginkgo biloba* L. and *Passiflora* sp.	
13	(undeclared composition)	0.4368
14	*Cynara scolymus*, *Fucus vesiculosus*, *Amorphophallus konjac*, *Rhamnus purshianai*, *Centella asiatica* and *Arctostaphylos uva-ursi*	0.3039
15	Advantra Z, *Fucus vesiculosus*, *Centella asiaticaI* and *Camelia sinensis* (green tea)	0.4688
16	Hydrochlorothiazide, *Fucus vesiculosus*, *Rhamnus purshiana*, *Cassia augustifolia*, *Centella asiatica*, ranitidine, triptophan and *Cordia Ecalyculata*	0.5608
17	Ágar-agar, vitamin E, vitamin C, phaseolamine, *Clorella*, goma guar, furosemide	0.4898
18	*Rhamnus purshiana*, *Cynara scolymus*, *Baccharis trimera*, *Fucus vesiculosus*, *Centella asiatica*,	0.3295
19	*Camelia sinensis* (green tea)	0.3153
20	*Centella asiatica*, *Cynara scolymus*, *Spirulina maxima*, *Baccharis trimera*, *Equisentum* sp., *Fucus vesiculosus*, *Passiflora* sp., *Cassia augustifolia* and *Rhamnus purshiana*	0.3809
21	*Amorphophallus konjac*, *Cynara scolymus*, hydrochlorothiazide, *Rhamnus purshiana*, *Centella asiatica* and *Ptychopetalum olacoides*	0.2917
22	*Garcinia cambogia*, Advantra Z, *Camelia sinensis* (green tea), benzocaine, *Amorphophallus konjac* and *Gelidium cartilagineum* (L.) Gaillon	0.6430
23	*Cordia ecalyculata* Vell	0.3739
24	*Citrus aurantium*, carnitine, *Camelia sinensis* (green tea), Chitosan	0.7876
25	*Cynara scolymusi*, *Centella asiatica*, *Garcinia cambogia*, *Equisentum* sp., *Rhamnus purshiana*, *Fucus*	0.4944

	vesiculosus, *Spirulina maxima* and *Gelidium cartilagineum* (L.) Gaillon	
26	*Allium sativum* 250 mg	0.3930
27	*Maytenus ilicifolia* 250 mg	0.3503
28	*Cynara scolymus* 500 mg	0.3984
29	*Valeriana officinallis* 225.75 mg	0.4698
30	*Cynara scolymus* 200 mg	0.7000
31	*Cynara scolymus* 200 mg	0.6169
32	*Valeriana officinallis* 250 mg	0.4018
33	*Equisentum* sp. 500 mg	0.5638
34	*Cynara scolymus* e *Cordia ecalyculata* Vell.	0.5067

Table 2. Retention time and MRM conditions for the studied adulterants.

Analyte	Ion Mode	Retention Time (min)	Precursor Ion (m/z)	Quantification (m/z)	Fragmentor (V)	Collision energy (eV)	Cell Accelerator Voltage (V)
Propranolol	+	2.702	260.1	116.2	96	14	5
Atenolol	+	0.337	267.2	190.1	110	11	5
Metoprolol	+	2.165	268.2	159.1	80	14	7
Nadolol	+	0.931	310.2	254.1	120	14	7
Captopril	+	2.459	218.0	116.0	95	5	5
Enalapril	+	3.149	377.2	234.2	110	10	7
Losartan	+	4.134	423.2	207.1	120	2	7
Valsartan	+	4.134	458.2	300.2	105	14	5
Furosemide	-	2.997	329.1	285.2	101	10	5
Hydrochlorothiazide	-	0.471	296.0	269.0	120	18	7
Chlorthalidone	-	2.687	337.0	146.1	120	13	5
Amiloride	+	0.402	230.0	171.1	95	19	7
Spironolactone	+	4.872	439.2	439.2	110	0	7

Table 3. Figures of merit of the UHPLC-ESI-MS/MS method for the determination of adulterants in herbal products.

Analyte	Linear Range (μg L^{-1})	LOD (μg L^{-1})	LOQ (μg L^{-1})	Precision Intra-day (%)[a]	Accuracy (%)
Propanolol	1.95 – 1000	0.30	1.00	0.16 – 2.30	98.11
Atenolol	1.95 – 1000	0.34	1.13	0.30 – 3.50	96.17
Metoprolol	1.95 – 1000	0.21	0.70	0.61 – 2.53	102.80
Nadolol	7.81 – 1000	0.02	0.08	0.52 – 2.56	85.88
Captopril	3.90 – 2000	0.64	2.14	0.39 – 2.33	90.55
Enalapril	1.95 – 1000	0.02	0.08	0.32 – 1.20	95.32
Losartan	1.95 – 500	0.05	0.18	0.08 – 3.29	99.54
Valsartan	9.76 – 1250	1.50	5.00	0.84 – 3.93	111.28
Furosemide	19.53 – 5000	0.47	1.57	0.17 – 3.19	80.56
Hydrochlorothiazide	9.76 – 5000	0.08	0.29	0.35 – 3.89	106.60
Chlorthalidone	19.53 – 5000	0.40	1.32	0.09 – 3.07	100.30
Amiloride	15.62 – 2000	0.14	0.47	0.01 – 1.35	86.63
Spironolactone	9.76 – 625	2.51	8.39	0.03 – 3.39	82.06

[a] Relative standard desviation (n = 3)

Table 4. Analyses of herbal products (n = 3) with declared synthetic drugs by UHPLC-ESI-MS/MS

Recommended daily dose for diuretics	Sample	Declared composition on the product label	Concentration determined (mg/capsule)	Instructions on the product label (capsules/day)	Ingested daily dose (mg/day)
Hydrochlorothiazide (12.5–25 mg/day)	7	*Fucus vesiculosus, Centella asiatica*, *Spirulina máxima*, *Passiflora* sp., *Rhamnus purshiana,* caffeine and hydrochlorothiazide	11.44±1.02	2	22.88
	16	Hydrochlorothiazide, *Fucus vesiculosus*, *Rhamnus purshiana*, *Cassia augustifolia*, *Centella asiática*, ranitidine, triptophan and *Cordia Ecalyculata*	19.99±0.27	2	39.98
	21	*Amorphophallus konjac*, *Cynara scolymus*, hydrochlorothiazide, *Rhamnus purshiana*, *Centella asiatica* and *Ptychopetalum olacoides*	2.62±0.03	2	5.24
Furosemide (20–40 mg/day)	9	*Cassia augustifolia*, *Fucus vesiculosus*, furosemide, *Rhamnus purshiana*, *Garcinia cambogia* and *Cyamopsis* sp.	19.22±1.57	2	38.44

	17	Ágar-agar, vitamin E, vitamin C, phaseolamine, *Clorella*, goma guar, furosemide	30.90±1.88	3	92.7

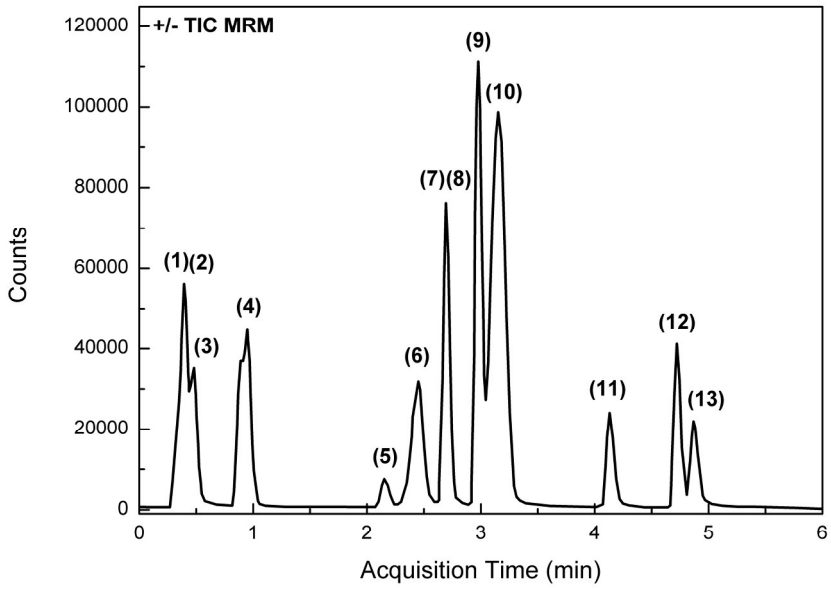


Figure 1: Total ion chromatogram (TIC) of 13 antihypertensive and diuretic adulterants (1) atenolol (1 mg L–1), (2) amiloride (1.0 mg L–1), (3) hydrochlorothiazide (5.0 mg L–1), (4) nadolol (1.0 mg L–1), (5) metoprolol tartrate (1.0 mg L–1), (6)captopril (2.0 mg L–1), (7) chlorthalidone (5.0 mg L–1), (8) furosemide (5.0 mg L–1), (9) propranolol hydrochloride (1.0 mg L–1), (10) enalapril maleate (1.0 mg L–1), (11) losartan (0.5 mg L–1), (12) spironolactone (0.625 mg L–1), (13) valsartan (1.25 mg L–1). Gradient elution: 15% methanol/0.1% acetic acid (0–0.5 min); 55% methanol/0.1% acetic acid (0.5–3.0 min); 100% methanol (3.0–6.0 min). Source parameters: 350 oC of gas temperature, 10 L/min of gas flow, 30 psi of nebulizer pressure, and 2000 V of capillary voltage.
204x144mm (300 x 300 DPI)

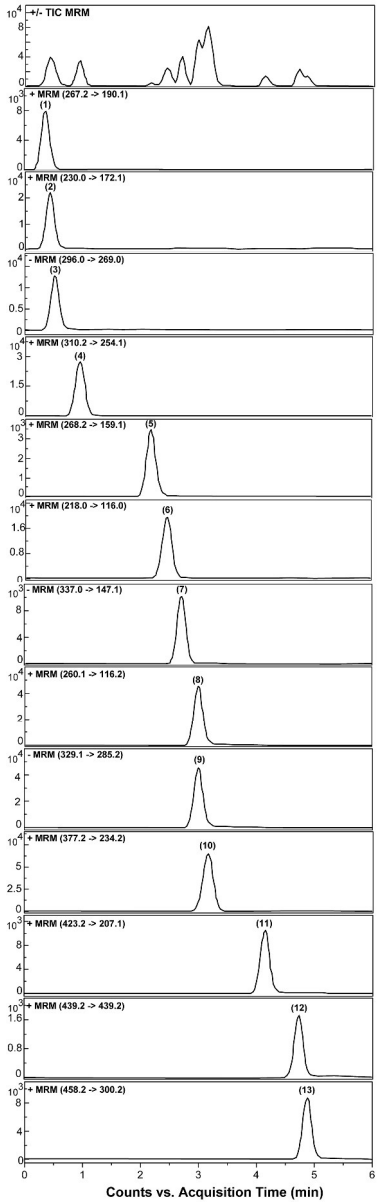


Figure 2: Extracted chromatogram (MRM) of 13 antihypertensive and diuretic as adulterants: (1) atenolol, (2) amiloride, (3) hydrochlorothiazide, (4) nadolol, (5) metoprolol tartrate, (6) captopril, (7) chlorthalidone, (8) furosemide, (9) propranolol hydrochloride, (10) enalapril maleate, (11) losartan, (12) spironolactone, (13) valsartan. Other conditions as described in Figure 1.
296x961mm (300 x 300 DPI)

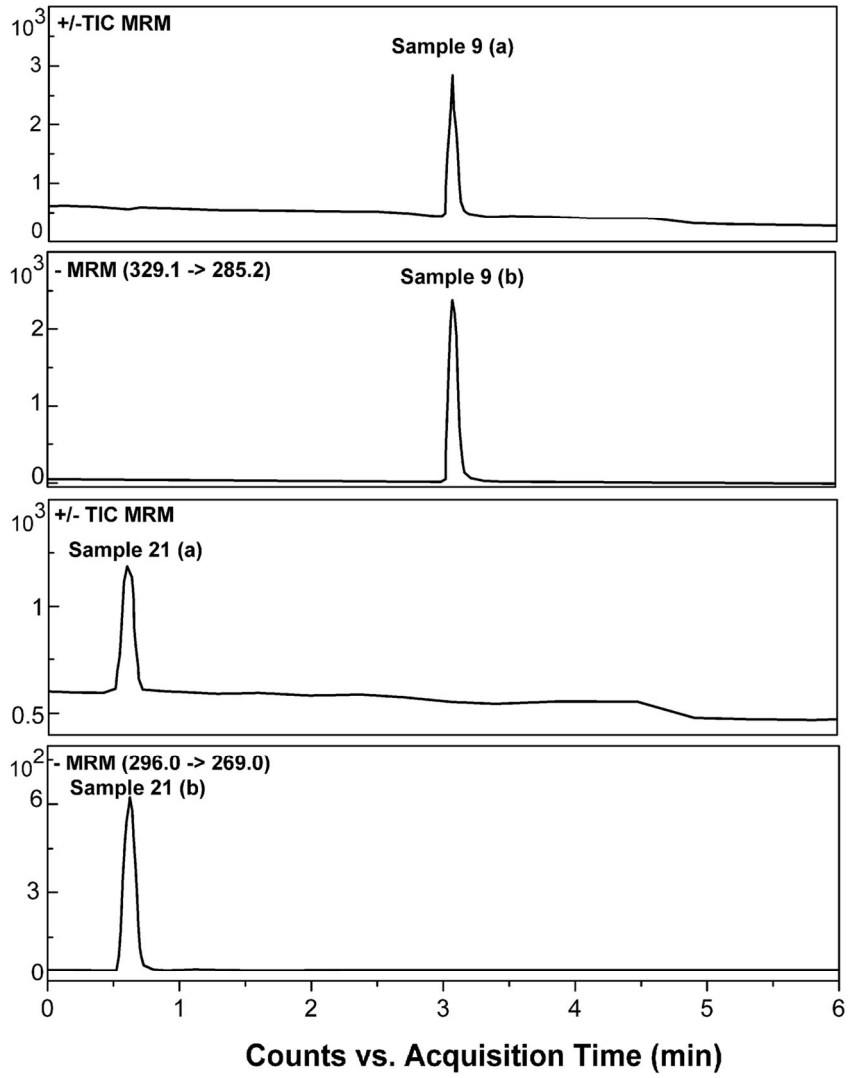
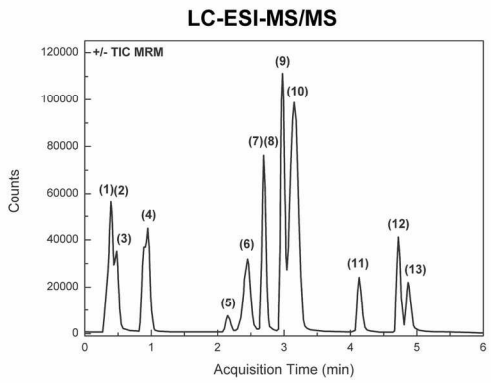


Figure 3: Chromatogram obtained from the analyses of samples 9 and 21 (Table 1). (a) Total ion chromatogram (TIC) with peaks detected in the retention time of furosemide (sample 9) and hydrochlorothiazide (sample 21); (b) Chromatogram extracted for transitions of furosemide (329.1>285.2) in sample 9 and hydrochlorothiazide (296.0>269.0) in sample 21. Other conditions as described in Figure 1.
129x139mm (300 x 300 DPI)



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