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ORIGINAL ARTICLES AND REVIEWS

A case of medicine in disguise: motion sickness patches sold as medical devices containing active pharmaceutical substances

Maria Cristina Gaudiano[1], Paola Bertocchi[1], Daniela De Orsi[1], Livia Manna[1], Eleonora Antoniella[1], Andrea Rodomonte[1], Angela Sorbo[2], Federica Aureli[1] and Monica Bartolomei[1]

[1]*Centro Nazionale Controllo e Valutazione dei Farmaci, Reparto Farmaci Chimici, Istituto Superiore di Sanità, Rome, Italy*
[2]*Dipartimento di Sicurezza Alimentare, Nutrizione e Sanità Pubblica Veterinaria, Istituto Superiore di Sanità, Rome, Italy*

Abstract

Introduction. A case study is reported on anti-motion sickness transdermal patches sold in the Internet, claiming to contain only natural ingredients but, actually, containing undeclared medicinal active substances.
The visual inspection of the samples evidenced many inconsistencies in secondary and primary packaging, missing of various legal information and a non-compliant "CE" mark.
Methods. The qualitative analysis was performed by liquid chromatography - high resolution mass spectrometry and the quantitative by liquid chromatography with diode array detector.
Results. The analyses evidenced the presence of the antihistaminic drug Diphenhydramine and of other active substances (Capsaicin, a transdermal absorption enhancer, and Diclofenac in traces, probably a contaminant from other productions of the same plant). Moreover, the presence of several trace elements, including those potentially toxic to humans, was assessed by ICP-MS analysis.
Conclusions. The case discussed is a new case of "medicines in disguise" never reported in literature, and shows the presence of tangible risks for public health.

Key words
- transdermal patch
- falsified medicines
- diphenhydramine
- liquid chromatography
- mass spectrometry
- ICP-MS

INTRODUCTION

Motion sickness is a common disturbance occurring in healthy people when they travel by car, plane, boat or train. This syndrome is thought to be caused by discordant signals coming from the vestibular and the visual systems [1, 2]. Several antiemetic drugs have been studied since the early 1940s, and since 1976 anticholinergic drugs and antihistamines (mainly acting as histamine H1 receptor antagonists and, sometimes, muscarinic receptor antagonists) were identified as excellent antiemetics. Diphenhydramine is an example of antihistamine drug also effective to prevent and treat nausea, vomiting and dizziness caused by motion sickness. This drug has been marketed as antihistaminic since 1946 but its antiemetic properties, which made it useful in the treatment of motion sickness, were discovered three years later, in 1949 [3]. As a H1 receptor antagonist, it can cause somnolence and sedation as side effects. To avoid these side effects, Dimenhydrinate, a combination drug of Diphenhydramine and 8-Chlorotheophylline, a stimulant drug and derivative of Theophylline, was developed. In the EU market, Diphenhydramine is used for motion sickness only by oral administration (tablets, capsules, gum, oral solution, dosage from 12.5 to 100 mg) and it is not recommended in young children, in elderly or during breastfeeding [4].

For the same therapeutic indication, transdermal patches containing Scopolamine (dosage 1.5 mg per patch) are successfully used in the USA. Transdermal Drug Delivery Systems (TDDS), also known as "patches," are dosage forms designed to deliver a therapeutically effective amount of drug across the patient's skin. They were developed in the 1970s and in 1979 the Food and Drug Administration (FDA) approved a

Address for correspondence: Maria Cristina Gaudiano, Centro Nazionale Controllo e Valutazione dei Farmaci, Reparto Farmaci Chimici, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy. E-mail: mariacristina.gaudiano@iss.it.

Scopolamine-containing patch. The therapeutic effect of the patch usually lasts from one to seven days, depending on the drug substance and the delivery system. The technology behind the Transdermal Drug Delivery System (TDDS) is critical to achieve good bioavailability, uniform blood drug levels, less side effects and a higher therapeutic effect with a lower dose compared with other delivery systems [5, 6].

Film forming solutions of Diphenhydramine for transdermal delivery have been studied [7], but in Europe no transdermal patches containing Diphenhydramine have been authorised by the competent authorities [8].

In the past years, the falsified medicine market has changed and expanded to other health products, such as food supplements, medical devices and cosmetics, where active pharmaceutical ingredients not declared on the label are fraudulently added [9-11]. In Europe the Official Medicines Control Laboratories (OMCLs) network coordinated by European Directorate for the Quality of Medicines & HealthCare (EDQM), name these products "medicines in disguise" [12] and invite the Member States to control these products on the national market with the aim of verifying the possible presence of undeclared active ingredients. The characteristic of these illegal products is that they do not claim to contain any active ingredients, but they generally claim to be "100% natural". Vegetal extracts and botanicals used for the preparation of natural health products such as herbal medicinal products, cosmetics or medical devices, can be naturally rich in minerals and trace elements (metallic and non-essentials) taken up by the plants during growth or as a result of environmental pollution from industrial and other anthropogenic activities [13, 14]. Inorganic impurities in medicinal products can originate from the manufacturing process, either added intentionally (e.g., reagents, ligands, catalyst) or resulting from contamination of raw materials or equipment employed during manufacturing. The presence of potentially toxic trace elements can be regarded as potential health concern for consumers' safety that should be warranted. Regulatory guidelines such as ICH Q3D [15] provides Permitted Daily Exposure (PDE) limits for those impurities considered having a higher potential safety risk (ICH Q3D).

In this study, we aimed to identify the nature and amount of any undeclared active pharmaceutical ingredients and toxic metal contamination of anti-motion sickness patches labelled as medical devices and claimed to be "herbal relief", marketed on e-commerce popular sites. The composition claim of these products includes *datura* plant, which also suggests the potential presence of Scopolamine as undeclared active drug substance with anti-sickness effect.

MATERIALS AND METHODS

Anti-motion sickness transdermal patches of four different brands were bought online on popular e-commerce web sites. Prior to instrumental analysis, samples were photographed and visually inspected for integrity of primary and secondary packaging, labelling (quality and coherence of information) and CE mark conformity. Sample information are summarised in *Table 1*. Batch numbers and expiry dates, where available, are also reported in *Table 1*.

Identification of active medicinal substances by liquid chromatography-mass spectrometry quadrapole time of flight (LC-MS Q-TOF)

All solvents and reagents used were of LC-MS grade by Sigma-Aldrich®. The presence of active medicinal substances contained in the patches was ascertained by liquid chromatography coupled to High Resolution Mass Spectrometry. Specifically, a screening analysis was carried out by a fast LC system, equipped with a diode array detector (Mod. 1290 Infinity) and a Dual ESI source MS Q-TOF detector, Mod. G6520B (all Agilent Technologies, Santa Clara, CA, USA). Data were processed with *MassHunter® Qualitative Analysis* version B.07.00. Identification of active pharmaceutical substances was obtained by MS and Auto MS/MS analysis in comparison with spectra contained in the MassHunter Forensic Toxicology Personal Compound Database and Library (ForTox PCDL B.07.01) and then confirmed in Target MS/MS against reference standard.

After removing the rear protective liner, each patch was divided in two halves for extraction. One-half was put in a small glass beaker containing 5 mL of methanol and the other one in 5 mL of water, both under magnetic stirring. After three hours, the extraction medium was analysed. The extraction was prolonged for further 6, 24 and 48 hours by adding 5 mL aliquots of fresh solvents each time. This procedure allowed checking solvent- and time-dependent differences in the extraction solutions. Sample extracts were diluted 1:10 with a solvent mixture of 0.1% formic acid in water/acetonitrile 50:50 v/v.

Diphenhydramine hydrochloride reference standard was purchased by Sigma-Aldrich®. Diphenhydramine standard solution for identification was prepared in methanol and then diluted in the same way as the sample extracts to obtain a final concentration of 0.01 mg/mL. All samples and standard solutions were filtered through polytetrafluoroethylene (PTFE) 0.2 µm filters before the analysis.

Chromatographic separation was achieved on a reversed-phase Zorbax Extend-C18 (2.1×50 mm, 1.8 µm) column by an in-house screening method consisting of a 15 minutes linear gradient elution from 100% of a mixture containing 0.1% formic acid in water/acetonitrile 95:5 v/v to 100% of a mixture containing 0.1% formic acid in water/acetonitrile 5:95 v/v. After the gradient, the system comes back to the initial condition in 1 minute and then remains in this condition for 4 minutes. Flow rate was 0.4 mL/min and the injection volume was 1 µL. Column temperature was set to 35 °C and the autosampler was thermostated at 15 °C.

MS analyses were carried out in both positive and negative ions mode; Auto MS/MS analysis was performed only in positive mode, since preliminary screening in MS mode did not show significant chromatographic peaks in negative mode. Finally, the presence of active medicinal substances was confirmed by Target MS/MS analysis by means of reference standards (purchased by Sigma-Aldrich®) in positive mode. MS parameters

Table 1
Results of the visual inspection on the primary and secondary packaging of patches

	Motion Sickness Patch 1	Motion Sickness Patch 2	Motion Sickness Patch 3	Motion Sickness Patch 4
Composition	*The abtract safflower, tall gastrodia tuber, sanchi, hairy datura flower, pinellia tuber, obtuseleaf cinnamon bark, frankincense, dahurian angelica root, borneol, etc.*	*The abstract of safflower, tall gastrodia tuber, hairy datura flower, pinellia tuber, obtuseleaf cinnamon bark, dahurian angelica root, frankincense, borneol, etc*	*Anti-sticking paper, matrix (the abstract of safflower, tall gastrodia tuber, sanchi, hairy datura flower, pinellia tuber, obtuseleaf cinnamon bark, frankincense, dahurian angelica root, borneol and medical pressure-sensitive adhesive), non-woven fabric*	*The abstract of safflower, tall gastrodia tuber, sanchi, hairy datura flower, pinellia tuber, obtuseleaf cinnamon bark, frankincense, dathurian angelican root, borneol, etc*
Notes on Batch number/expiry date	Inconsistency between the batch number and expiry date reported in primary and secondary packaging. Patches with different batch number/expiry date are in the same box	Batch number and expiry date are not reported	Batch number is reported as a date	Batch number is not reported (only two sequences of numbers are reported, probably related to the Manufacturing date and Expiry date)
Batch number/ expiry date (numbers are reported as in the samples)	*Lot. No.20181202/ Exp.: 20211001* and *Lot. No.20181002/Exp.:20211201* in the same packaging but on the secondary packaging is reported: *EXPIRY DATE: 01/AUG/2021*		*Lot No: 2018.12.16 Exp: 2021.12.15*	*20190702 20220701*
Presence of CE/ FDA Mark	"CE Certified by European Standard" and "FDA" marks are reported on the packaging. "CE" mark is counterfeit[1]			A "CE European Standards" mark is on primary packaging. "CE" mark is counterfeit[1]
Inconsistencies and claims	The number of patches reported on the secondary packaging is inconsistent with the real number "Long effect: 72 hours" "Safe and Effective"	Secondary packaging is in English, in the primary packaging pictograms and ideograms are reported Long effect: 72 hours Warnings include "not used by pregnant woman and kids under aged 4". "No side effects"	The packaging reports "100% herbal relief". Long effect: 72 hours Warning includes "one/two patches for time" and "not used by pregnant woman and kids under aged 4".	Absence of secondary packaging. Long effect (inconsistency: 48 reported in one face and 72 hours in the other one of the sachet)
Manufacturer/ Brand	The name of the Manufacturer is slightly different from the name reported in the logo. No information on the Country and address of the Manufacturer	The Brand reported in the secondary packaging is different from that reported in the primary packaging. No information on the Manufacturer name and address	No information on the address of the Manufacturer	No information on the Manufacturer name, address and Country of production. Only a logo is reported

[1]"CE" marking does not respect the distance between C and E of the original mark.

were: Fragmentor 100 V, Nitrogen temperature 300 °C, Drying gas 10 L/min, Nebulizer 40 psig, VCap 4000 V. Collision offset voltage (in Auto and Target MS/MS experiments) was 20 V. In Auto MS/MS experiments, the maximum precursors for cycle were 3. Mass range was 100-1200 Da in MS analysis and 50-1200 Da in MS/MS analysis.

Quantitative analysis of targeted active medicinal substances

All solvents and reagents were of high performance liquid chromatography (HPLC) grade. At least two patches of each sample were analysed. Samples extraction was optimised as follows: after removing the rear protective liner, each patch was cut in many parts (at least 5) and placed in a small glass beaker (closed with a petri disc) containing 10 mL of methanol. Two small magnetic stirring bars were used to prevent sticking of the patch on the bottom and the walls of the beaker, and to increase the solvent-patch surface contact. The solution was stirred for three hours, then the extraction medium was collected and analysed for the quantification of Diphenhydramine and Diclofenac (when detected). The same extraction procedure was repeated until the chromatographic signal of the analytes was negligible, i.e., at increasing times up to at least 72 hours (3, 6, 24, 48, 72 hours). For extracts containing higher quantity of Diphenhydramine (milligrams), the quantity of solvent added was increased up to 50 mL, to obtain the complete extraction from the patch.

The quantitative determination of Diphenhydramine and Diclofenac that had been previously identified in patches, was performed by an Agilent HPLC 1100 series equipped with a diode array detector (mod. 1260 Infinity). HPLC method for quantitative assay of Diphenhydramine was the one described in Euro-

pean Pharmacopoeia Diphenhydramine hydrochloride monograph for the determination of related substances [16] with slight modifications. Briefly, Diphenhydramine was eluted in isocratic conditions with a mobile phase containing 35/65 v/v acetonitrile/potassium dihydrogen phosphate buffer (5.4 g/L at pH = 3.0) (A) for 8 min as prescribed, then a gradient step, up to 90/10 v/v acetonitrile/mobile phase A, was added to elute potentially interfering molecules observed during LC-MS screening analysis. Chromatographic column was a Symmetry C8 250 x 4.6 mm, 5 µm particle size, the flow rate was 1.2 mL/min, detection wavelengths were at 220 and 254 nm, and the injection volume was 10 µL.

HPLC method for quantitative determination of Diclofenac was obtained from literature [17] Chromatographic separation was performed with an isocratic elution (methanol: phosphate buffer pH 2.5 70:30 v/v) and UV detection at 275 nm by using a Zorbax RX C8, 150 mm x 4,6 mm, 5 µm particle size column. Flow rate was 1 mL/min and the injection volume was 20 µL.

Trace elements analysis

Sample manipulations were carried out in clean room conditions under a laminar flow box (Spetec GmbH, Erding, Germany). Analytical grade HNO_3 67% w/w (Romil, Cambridge, UK), H_2O_2 30% w/w (Romil, Cambridge, UK) and HF 40% w/w (PanReac, Barcelona, Spain) were used for sample digestion. Ultrapure water obtained by a Milli-Q system (Zeener UP 900 Water Purification System, Human Corporation, Texas, United States) was employed for sample preparations and dilutions. Certified stock solutions of 1000 mg/L As, Co, Cr, Cd, Cu, Mo, Pb, Ni, Rh, Sb, Tl, Zn, and Rh (as internal standard) (High-Purity Standards, North Charleston, South Carolina, United States) were used to build the calibration curve for total elements' quantification by inductively coupled plasma mass spectrometry (ICP-MS). All standard solutions were daily prepared by diluting the stock solution in 1% v/v HNO_3. Complete sample dissolution was accomplished by mean of high temperatures and microwave irradiation system with mixtures of HNO_3, H_2O_2 and small amounts of HF, added in order to ensure complete sample decomposition. From 0.05 to 0.2 g of protective liner-free samples were digested by closed vessel microwave system (UltraWAVE, Milestone, FKV, Bergamo, Italy) with 1 mL H_2O + 3 mL HNO_3 + 1mL H_2O_2 +0 .5mL HF using the following temperature program: up to 85 °C (ramp 20 °C/min) and stabilization for 8 min; up to 145 °C (ramp 20 °C/min) and stabilization for 5 min; up to 200 °C (ramp 22 °C/min); hold at 200 °C for 20 min before cooling down. Each sample was digested in duplicate and digestion blanks were run in parallel.

Determination of total elements content was carried out by a NexION 350D ICP-MS (Perkin-Elmer, Shelton, CT, USA) equipped with a Meinhard micro nebulizer, a quartz cyclonic spray chamber and Pt cones. The instrument operated at 1600 W in standard mode with Argon as carrier gas and in collision mode (KED) with He (purity 4.9, Sapio, at 4.1 ml/min) filling the cell. Analytical masses were as follows: ^{75}As, ^{59}Co, ^{111}Cd, ^{112}Cd, ^{114}Cd, ^{63}Cu, ^{65}Cu, ^{95}Mo, ^{98}Mo, ^{206}Pb, ^{207}Pb, ^{208}Pb, ^{121}Sb, ^{123}Sb, ^{203}Tl, ^{205}Tl in standard mode and ^{52}Cr, ^{53}Cr, ^{60}Ni, ^{62}Ni, ^{64}Zn, ^{66}Zn in KED mode. The ICP-MS measurement conditions were optimized daily to provide the highest intensity using standard built-in software procedures (Syngistix for ICP-MS, Version 2.3). Quantitative measurements were carried out using the standard addition approach (calibration range 1-50 µg/L). Digestion blanks were analysed in parallel with samples belonging to the same analytical batch. The final concentration of the chemical elements was obtained by subtracting the blank signal to the sample signal for each analyte.

Due to the lack of suitable certified reference materials, the trueness of the measurements was evaluated by spiking samples with known amounts of analytes. The recovery rates turned out to be satisfactory, ranging from 91.6% to 116.9%.

Instrumental limits of detection (LDs) were calculated following the 3σ criteria, and were in the range 0.008-0.28 µg/g.

RESULTS

Visual inspection

All the samples consisted of round brown patches of variable diameters (20 mm Patches 1 and 3, 30 mm Patches 2, 4) contained in sachets as primary packaging. Sachets (10, 20 or 30) were contained in a card box (secondary packaging) except for sample "Patch 3" that was sold with no secondary packaging. All the sachets were intact. All the information were reported on the sachet and on the card box, when available. No leaflet was included for all samples. Descriptive information was reported in narrative form in English language, except for sachets of Patch 2 that reported only pictograms and ideograms. The composition reported on the label is given in *Table 1*, for each patch. The same ingredients were listed for all samples, with few differences in the description of Patch 3. All the samples reported the same indications: "relieve the vomiting, nausea, dizziness, anorexia, and other symptoms resulted from sickness of cars, ships, airplanes, trains and other means of transport". Instructions of use were the same for all the samples: site of application abdomen or behind one ear, ten minutes before the travel, long lasting 1-3 days. Patch 1 requires using one patch per time. Patch 3 one/two patch per time, "according to your body conditions". Warning sentences are quite different: Patch 2 and 3 reported the same peculiar indications: "Not used by pregnant women and kids under aged 4" and "Not recommended to use by poorly surgery body".

Visual inspection of the samples highlighted many anomalies in the labelling, suggesting an illegal production. Punctuation and grammatical/translation errors (e.g., "abstract" – instead of extract – "by poorly surgery body"); no botanic names were reported in the declared composition, so it was impossible to assess exactly the characteristics of the extracts used; in some patches (specifically, 1-3) the list of ingredients ended with "etc.".

The results of the visual inspection showed that many legal information, such as the name of the Manufacturer (absent in two cases) and the Manufacturer's ad-

dress (absent in all cases) were missing on the packaging. Inconsistencies concerning the number of patches contained in the box or between the batch number and expiry date reported in the primary and secondary packaging were observed, suggesting poor control during manufacturing or a potential risk of falsification. In one case (Patch 4) the secondary packaging (card box) was different from that reported in the primary one. Moreover, the "CE" mark, which means "European Conformity", was followed by the definition "Certified by European Standard" or "European standards" and in one case the mark was evidently false (the typographic font of C and E and the distance between them did not comply with the law requirements) [18]. *Figure 1* reports the photographic image of the patch with emphasis on inconsistencies.

Identification of active medicinal substances by LC-MS Q-TOF

MS qualitative analysis showed the presence of Diphenhydramine and its related impurity desmethyldiphenydramine (Eur. Ph. Impurity A) in all patches. Moreover, in three patches (Patch 1, 2 and 3) the presence of Diclofenac was also detected. Diphenhydramine and Diclofenac identification was confirmed by MS/MS in comparison with a commercial reference standard. *Figure 2* shows (for Patch 1) the extracted ion chromatographic peak, the mass spectrum and the Auto MS/MS spectrum (reporting the match in database for the identification of Diphenhydramine). Other undeclared constituents, such as Capsaicin and Dihydrocapsaicin were found in Patch 2 by Auto MS/MS analysis, with a high identification score with spectral database, suggesting cross-contamination problems in production. Ultimately, the presence of Scopolamine, that was suspected to be contained in the patch as an undeclared active drug substance with anti-sickness effect, was not confirmed by the results obtained.

Quantitative analysis of targeted active medicinal substances

Quantitative extraction was a critical point due to a very low patch-to-patch reproducibility, not only among patches of different batches, but also among patches of the same lot. Quantities lower than milligrams/patch of Diclofenac were found, probably due to contamination related to non-GMP compliant manufacture of different kind of products. On the other hand, a content of Diphenhydramine, ranging from 0.5 mg to 3 mg per patch was found. The results of the quali-quantitative analysis showed higher quantities of Diphenhydramine in Patch 3 and Patches 2 than in Patch 1 and Patch 4. It should be noted that for Patch 3 a 72-hour time-point was not sufficient to obtain negligible chromatographic signal of Diphenhydramine. Notwithstanding the extraction time was extended up to 210 hours, a steady state could not be reached and quantities in the order of milligrams were still recovered.

Determination of total elements content

Results obtained for total elements content are depicted in *Table 2*, where the mean value and the standard deviation (SD) associated with the instrumental measurements and digested samples (n = 4) is reported for each analyte.

Three groups of elements were considered accord-

Figure 1
Photographic image of a patch with emphasis on inconsistencies reported in the paper.

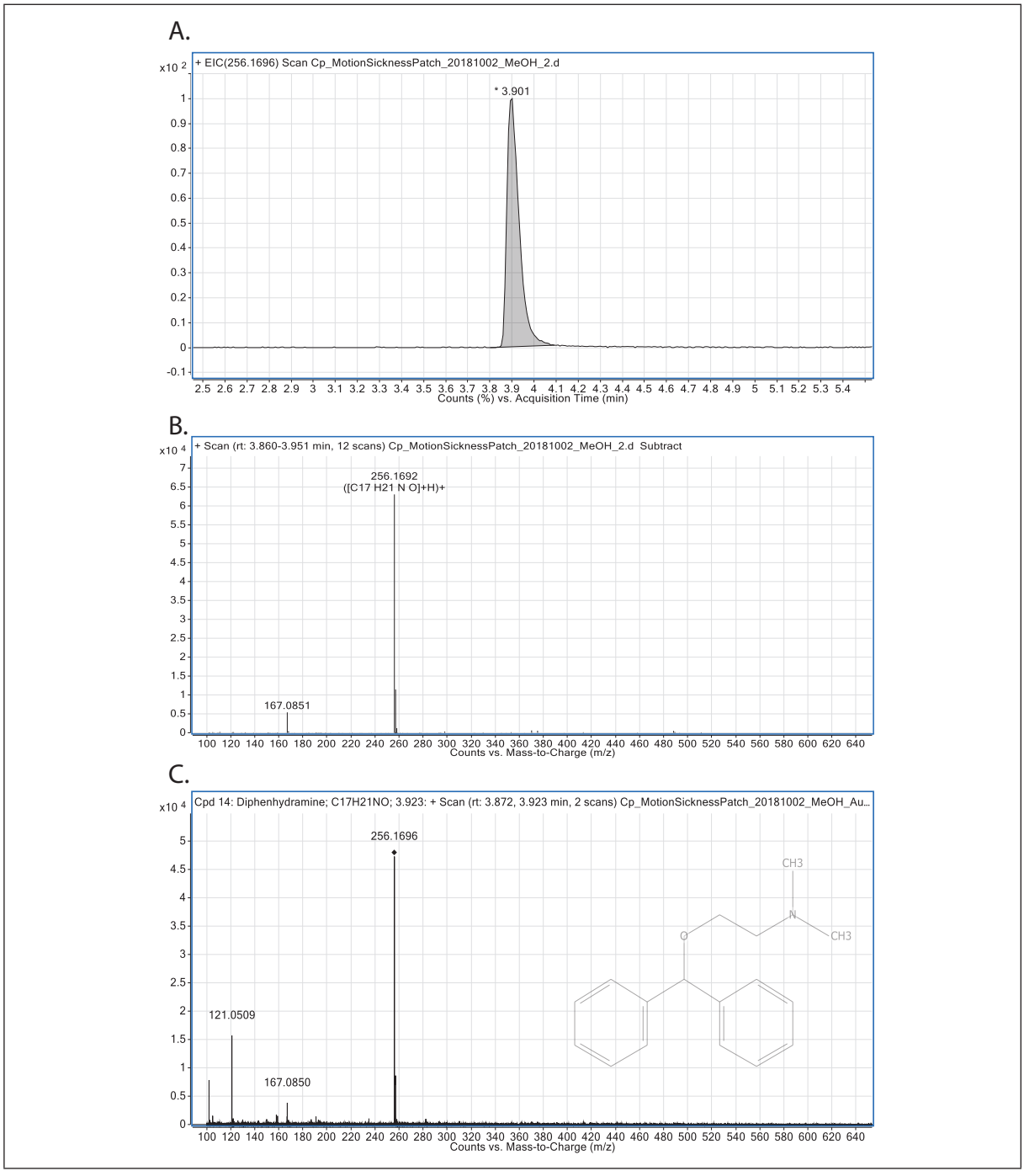

Figure 2
Extracted ion chromatographic peak of Diphenhydramine (panel A), mass spectrum of Diphenhydramine (panel B) and Auto MS/MS spectrum reporting the match in database for the identification of Diphenhydramine (panel C) of a sample of Patch 1.

ing to their ICH classification [15]: class 1 comprising known human toxicants such as As, Cd, Pb; class 2 including elements generally considered as route-dependent human toxicants such as Ni, Co, Tl and class 3 with all the other elements.

All samples showed low but detectable concentrations of As, Cr, Cu, Mo, Pb, Ni, Zn, Sb; on the other hand, Tl was systematically below the LD in all the analysed samples, Cd was below LD in two out of four samples and Co was below LD only in sample 2. The lowest detected amount of As (0.02 μg g^{-1}), Cr (0.66 μg g^{-1}), Cu (0.45 μg g^{-1}), Mo (0.05 μg g^{-1}) and Pb (0.13 μg g^{-1}), were found in sample 2, Ni (0.38 μg g^{-1}), Zn (4.96 μg g^{-1}) and Cd (0.037 μg g^{-1}) in sample 4, Sb (42.5 μg g^{-1}) in sample 1 and Co (0.09 μg g^{-1}) in sample 3, respectively. The overall elements content in samples followed the order 1~3 >4>2 for class 1, 1~4 >3~2 for class 2, and 2>4>3>1 for class 3, mainly due to the contribution of Sb (42.5-128.2 μg g^{-1}). The results highlight elemental concentration range in samples from different suppli-

Table 2
Distribution of trace elements and dermal exposures calculated according to ICH in transdermal systems selected for this study

	PDE	Sample Patch 1		Sample Patch 2		Sample Patch 3		Sample Patch 4	
	µg day^{-1}	µg g^{-1}	µg day^{-1}	µg g^{-1}	µg day^{-1}	µg g^{-1}	µg day^{-1}	µg g^{-1}	µg day^{-1}
As	15	0.208±0.012	0.019	0.021±0.001	0.002	0.187±0.019	0.012	0.103±0.003	0.017
Co	50	0.728±0.021	0.066	<LD	NA	0.089±0.006	0.006	1.298±0.024	0.214
Cr	11000	0.642±0.015	0.058	0.664±0.014	0.060	1.850±0.099	0.117	0.776±0.012	0.128
Cd	5	0.043±0.002	0.004	<LD	NA	<LD	NA	0.037±0.001	0.006
Cu	3000	0.808±0.049	0.073	0.450±0.029	0.040	0.520±0.023	0.033	0.801±0.056	0.132
Mo	3000	0.176±0.004	0.016	0.052±0.001	0.005	0.114±0.001	0.007	0.039±0.002	0.006
Pb	5	1.986±0.075	0.179	0.133±0.007	0.012	2.153±0.086	0.136	1.731±0.087	0.286
Ni	110	0.569±0.021	0.051	0.772±0.065	0.069	0.619±0.054	0.039	0.381±0.008	0.063
Sb	1200	53.40±3.03	4.81	128.2±9.12	11.54	78.47±2.28	4.94	96.92±2.68	15.99
Tl	8	<LD	NA	<LD	NA	<LD	NA	<LD	/
Zn	NA	8.68±0.52	NA	36.35±1.76	NA	8.03±0.42	NA	4.96±0.24	/

NA: not applicable; LD: limits of detection. ICH: International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use.

ers (1-4) spanning a 10-30 fold variation range within elements of class 1, a 4-15 fold range for class 2, and a narrower 2-7 fold range for elements of class 3.

DISCUSSION

Undeclared active ingredients

Anti-motion sickness patches claiming only natural ingredients and freely marketed on e-commerce web sites, actually contain active drug substances undeclared on the label. Visual inspection showed many inconsistencies and errors in the labelling, indicating signs of a potential falsification or at least very poor quality in production. Appearance of all the patches was very similar, labels reported the same composition and often the same typing errors. All the analysed patches contained Diphenhydramine: after over 72 hours extraction Patch 3 still contained measurable quantities of Diphenhydramine suggesting a different matrix able of a longer lasting action; three patches showed low quantity of Diclofenac, suggesting a cross-contamination due to a non-GMP manufacture of different products; Capsaicin was identified in Patch 2.

Diphenhydramine is an antihistamine with anticholinergic and sedative effects. Commercial medicinal products containing Diphenhydramine are legally placed on the market as tablets, capsules, oral solutions, intramuscular or intravenous injections or pharmaceutical forms for topical use (creams) whereas there are not transdermal patches containing Diphenhydramine authorised in the EU. The authorised dosage of Diphenhydramine for oral use for motion sickness ranges from 12.5 to 100 mg in the EU. It is well known that transdermal patches require even lower dosages to achieve a therapeutic effect [5]. Medicines containing Diphenhydramine are contra-indicated in people with a specific hypersensitivity to Diphenhydramine and similar antihistamine molecules, in pregnancy and during breastfeeding, in patients with glaucoma and in people taking antidepressant drugs. Diphenhydramine has additive effects with alcohol that may jeopardise consumers health if they are not properly informed [19]. Capsaicin found in Patch 2 is an active medicinal substance generally used as topical analgesic and as patch in the treatment of neuropathic pain [20, 21]. Furthermore, its properties to promote skin permeability in transdermal drug delivery were reported [22]. The European Pharmacopoeia contains monographs for "Capsici fructus" and "Capsicum Oleoresin". The European Scientific Cooperation on Phytotherapy (ESCOP) has classified "Capsici fructus" as an herbal medicinal product. According to the outcome of the Manual of Borderline "a plaster with Capsaicin may not be qualified as a medical device" [23]. The undeclared presence of active pharmaceutical ingredients in patches claimed to contain only natural ingredients makes these products dangerous to health. Furthermore, transdermal patch is a sophisticated drug delivery system, which is difficult to formulate. It requires specialized manufacturing process/equipment to meet specific pharmacological and functional characteristics. The uncontrolled production of transdermal patches does not ensure these characteristics, leading to a device that could release the active substance too fast or, on the contrary, too slow, or leading to a rapid degradation of the active ingredient due to interaction with the patch matrix. Finally, in this formulation the choice of a non-toxic adhesive matrix that is suitable for dermal use should be carefully evaluated. In products freely marketed, these characteristics are not controlled and can cause allergic reactions and pose a health hazard.

Trace elements

Vegetal extracts and Botanicals used for the preparation of herbal medicinal products, cosmetics or medical devices can be rich in trace elements [24]. The distribution tendency of trace elements, specifically those of class 1 and 2, in the samples selected for this study cover a wide range of concentration notwithstanding a similar composition claimed on the product label. As pointed out in the visual assessment, the samples se-

lected for this study are characterized by inadequate or incomplete description of composition; therefore, exact taxonomic botany of components could not be ascertained. Possible explanations of the distribution tendency might be related to different plant origin, environmental factors [25] and production processes, including adulteration with active pharmaceutical ingredients. Trace elements and metals can in fact be regarded as impurities in pharmaceutical industry originating from elements intentionally added (e.g., reagents, ligands catalyst) or not intentionally added (e.g., contamination originating from the manufacturing equipment or raw materials) to the products [26, 27]. Over the last decades, trace elements have been studied in natural health products where undeclared or excessive active pharmaceutical ingredients were found [27, 29-31]. As (14.6 ppm), Pb (1.05-75 ppm), Cd (0.24-39 ppm), Ni (2.33-45 ppm), Cr (1.68-110 ppm), Cu (0.24-28 ppm), Mo (2.56-45.2 ppm) Tl (0.037-2.07 ppm), and Co (0.038-9.55 ppm) were found at higher levels than those found in the present study, but none of these investigations specifically focused on transdermal systems. On the other hand, Zn levels (13-80 ppm) were comparable whereas Sb concentrations (0.79-2.13 ppm) were considerably lower than those found in this study, likely due to the possible contribution of the non-woven substrate used for the production of the transdermal system [31, 32].

Provisional safety assessment

ICP-MS results were used to carry out a safety assessment for each sample calculating dermal exposure by assuming the use of one or two patches per time, as per indications on the label. Due to the presence of the active pharmaceutical ingredients Diphenhydramine and Diclofenac among others, the selected samples were regarded as medicinal products [33]. Therefore, the assessment was carried out following the principles of ICH Q3D guideline set out under the EU pharmaceutical legislation. Health based exposure limits are expressed as permitted daily exposure (PDE, mg/day) for all the studied elements. Element specific dermal PDEs were established by Bouvier et al. based on the oral PDEs set in ICH Q3D [34, 35]. Results are presented in *Table 2*. Dermal absorption of trace elements is typically low and dependent upon the properties of the skin, the anatomical site, the physical-chemical properties of the mixture and the characteristics of the application [13, 14, 36, 37]. The highest estimated daily exposures were found for Sb (Class 3) and Pb (Class 1), however for all samples the calculated cutaneous concentrations were below 10% of the estimated PDEs.

Among the studied elements, nickel, cobalt, and chromium are the most important contact human allergens, with nickel representing the leading contact allergen in most industrialized countries worldwide [38, 39]. Samples were evaluated for sensitization from Ni and Co, according to the approach developed by Lim *et al*. based on sensitization quantitative risk assessment [37]. For Chromium the sole PDE was considered appropriate (ICH). Therefore, transdermal systems were treated as leave-on cosmetic products and only single mineral exposure was considered. Dermal sensitization is a threshold-based phenomenon [40, 41], the % concentration of elements in a product type is acceptable if the Consumer Exposure Level (CEL) is lower than the Acceptable Exposure Level (AEL) [37]. The assessment reported in *Table 3* shows that AEL/CEL ratios were higher than 1, therefore the compounds were not indicative of a potential skin sensitizer. It is important to stress that this study only provides a snapshot of elemental levels in a limited number of samples that may not reflect the elemental content variability of transdermal systems. Actually, the assumptions made in this study for transdermal systems may represent a source of uncertainty to the proposed assessment. A more refined exposure assessment taking into account other sources of exposures (e.g., food) or the study of combined exposure to chemical sensitizers, is also recommended, specifically for children and other vulnerable groups.

CONCLUSIONS

This case study concerning falsified anti-motion sickness transdermal patches, proved for the first time that these products, claiming only natural ingredients and freely marketed on commercial web sites, actually contain active drug substances. These products are claimed as medical devices and some of them reported a falsified CE mark on the packaging. All of the analysed products reporting only natural ingredients and claiming to be "100% natural relief" in the composition contained some milligrams per patch of Diphenhydramine, an active medicinal substance. Transdermal patches containing Diphenhydramine are not authorised in the EU. Therefore, it is not possible to know whether the quantity of Diphenhydramine found in the patches can have a therapeutic effect, but Diphenhydramine was considered a candidate for non-invasive transdermal

Table 3
Sensitization assessment for Ni and Co

	Ni			*Co*		
	CEL (µg/cm²/day)	*AEL* (ug/cm²)*	*AEL/CEL*	*CEL (µg/cm²/day)*	*AEL* µg/cm²*	*AEL/CEL*
Sample Patch 1	4.1E-03	1.34	328	5.2E-03	1.04	199
Sample Patch 2	2.5E-03	1.34	545	2.0E-03**	1.04	530
Sample Patch 3	3.1E-03	1.34	432	4.5E-04	1.04	2329
Sample Patch 4	2.2E-03	1.34	602	7.6E-03	1.04	137

*Reported on Lim *et al.*, 2018 [37]. **LD/2 was used for calculation. LD: limits of detection. CEL: Consumer Exposure Level; AEL: Acceptable Exposure Level.

delivery system [7]. Overall, patches sampled in this study can actually be considered "medicines in disguise" freely marketed on the internet and represent a potential health risk to end-users – including children over 4 – targeted with one or two patches per time for a long time (1-3 days) according to the label indications.

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Author's contribution

MCG: LC MS measurements and elaboration of results, drafting manuscript. LM: LC MS measurements and elaboration results. PB, EA: quantitative HPLC analysis. DDO: idea proposal, quantitative HPLC analysis. AR: statistical evaluation of results, revision of the manuscript. AS, FA: ICP-MS analysis and drafting manuscript. MB: coordination and critical revision of the study, revision of the manuscript.

Conflict of interest statement

None.

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REFERENCES

1. Golding JF, Gresty MA. Pathophysiology and treatment of motion sickness. Current Opinion in Neurology. 2015;28:83-8. Available from: https://journals.lww.com/00019052-201502000-00015.
2. Bertolini G, Straumann D. Moving in a moving world: a review on vestibular motion sickness. Front Neurol. 2016;7. Available from: http://journal.frontiersin.org/Article/10.3389/fneur.2016.00014/abstract.
3. Emanuel MB. Histamine and the antiallergic antihistamines: a history of their discoveries. Clin Exp Allergy. 1999;29:1-11. Available from: http://doi.wiley.com/10.1046/j.1365-2222.1999.00004.x-i1.
4. Sanger GJ, Andrews PLR. A History of drug discovery for treatment of nausea and vomiting and the implications for future research. Front Pharmacol. 2018;9:913. Available from: www.frontiersin.org/article/10.3389/fphar.2018.00913/full.
5. Pastore MN, Kalia YN, Horstmann M, Roberts MS. Transdermal patches: history, development and pharmacology. Br J Pharmacol. 2015;172:2179-209. Available from: https://onlinelibrary.wiley.com/doi/10.1111/bph.13059.
6. Mali AD, Bathe R, Patil M. An updated review on transdermal drug delivery systems. Int J of Adv in Scient Res. 2015;1:244. Available from: http://ssjournals.com/index.php/ijasr/article/view/2243.
7. Baby A, Shivakumar HN, Alayadan P. Formulation and Evaluation of Film Forming Solution of Diphenhydramine Hydrochloride for Transdermal Delivery. IJPER. 2022;56:43-9. Available from: https://ijper.org/article/1529.
8. Public data from Article 57 database European Medicines Agency (europa.eu). Available from: www.ema.europa.eu/en/human-regulatory/post-authorisation/data-medicines-iso-idmp-standards/public-data-article-57-database.
9. Bartolomei M, Gaudiano MC, Manna L, Caldarone C, Romanini L, Bertocchi P, et al. Integratori alimentari contraffatti nel mercato europeo i risultati del progetto ASKLEPIOS. Roma: Istituto Superiore di Sanità; 2019. (Rapporti ISTISAN, 19/10).
10. Mustazza C, Gaudiano MC, Borioni A, Valvo L. The evolution of the illegal market of falsified medicines and the experience of the Italian OMCL: from control to research. Ann Ist Super Sanità. 2018;54:(4):267-9. doi: 10.4415/ANN_18_04_01
11. Council of Europe.European Directorate for the Quality of Medicines & Healthcare. Testing of falsified/illegal medicines. Market surveillance Studies on Suspected Illegal Products (MSSIP) within the Network. Available from: www.edqm.eu/en/testing-falsified-illegal-medicines.
12. Council of Europe-General European OMCL Network (GEON). General Document PA/PH/OMCL (06) 81 R8 An "aide-mémoire" for the testing of suspected illegally traded and falsified medicines. Strasbourg: OMCL; 2019.
13. Tarnowska M, Briançon S, Resende de Azevedo J, Chevalier Y, Bolzinger M-A. Inorganic ions in the skin: Allies or enemies? International Journal of Pharmaceutics. 2020;591:119991. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0378517320309765.
14. Bocca B, Pino A, Alimonti A, Forte G. Toxic metals contained in cosmetics: A status report. Regul Toxicol Pharmacol. 2014;68:447-67.
15. European Medicines Agency-EMA. ICH Guideline Q3D (R1) on elemental impurities Q3D(R1) EMA/CHMP/ICH/353369/2013. 2019. Available from: www.ema.europa.eu/en/documents/scientific-guideline/international-conference-harmonisation-technical-requirements-registration-pharmaceuticals-human-use_en-32.pdf.
16. EDQM Council of Europe - European Pharmacopoeia 10th Edition-Diphenydramine Hydrochloride Monograph 01/2016:0023. Strasbourg, France; 2016.
17. Rubim AM, Rubenick JB, Laporta LV, Rolim CMB. A simple method for the quantification of diclofenac potassium in oral suspension by high-performance liquid chromatography with UV-detection. Braz. J Pharm Sci. 2013;49(3):589-97. Available from: https://doi.org/10.1590/S1984-82502013000300021.
18. European Parliament. Regulation (EU) 2017/745 of the European Parliament and of the Council of 5 April 2017 on medical devices, amending Directive 2001/83/EC, Regulation (EC) No 178/2002 and Regulation (EC) No 1223/2009 and repealing Council Directives 90/385/EEC and 93/42/EEC-Annex V. 2017.
19. Weathermon R, Crabb DW. Alcohol and medication interactions. Alcohol Res Health. 1999;23:40-54.
20. Fattori V, Hohmann M, Rossaneis A, Pinho-Ribeiro F, Verri W. Capsaicin: Current Understanding of Its Mechanisms and Therapy of Pain and Other Pre-Clinical and Clinical Uses. Molecules. 2016;21:844. Available from: www.mdpi.com/1420-3049/21/7/844.
21. Wohlrab J, Neubert RHH, Heskamp M-L, Michael J. Cutaneous Drug Delivery of Capsaicin after in vitro Administration of the 8% Capsaicin Dermal Patch System. Skin Pharmacol Physiol. 2015;28:65-74. Available from: www.karger.com/Article/FullText/362740.
22. Das A, Ahmed AB. Natural permeation enhancer for

transdermal drug delivery system and permeation evaluation: a review. Asian J Pharm Clin Res. 2017;10:5. Available from: https://innovareacademics.in/journals/index.php/ajpcr/article/view/19389.
23. European Commission. Manual on borderline and classification in the community regulatory framework for medical devices Version 1.22. 2019.
24. Prasad MNV. Trace elements as contaminants and nutrients: consequences in ecosystems and human health. Hoboken, NJ: Wiley; 2008.
25. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 2014;4. Available from: http://journal.frontiersin.org/article/10.3389/fphar.2013.00177/abstract.
26. Ernst E. Toxic heavy metals and undeclared drugs in Asian herbal medicines. Trends Pharmacol Sci. 2002;23:136-9. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0165614700019726.
27. Balaram V. Recent advances in the determination of elemental impurities in pharmaceuticals: Status, challenges and moving frontiers. Trends Analyt Chem. 2016;80:83-95. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0165993615300042.
28. Ozdemir B, Sahin I, Kapucu H, Celbis O, Karakoc Y, Erdogan S, et al. How safe is the use of herbal weight-loss products sold over the Internet? Hum Exp Toxicol. 2013;32:101-6. Available from: http://journals.sagepub.com/doi/10.1177/0960327112436407.
29. Wong PHB, Harnett JE, Clases D, Wheate NJ. An Analysis for Adulteration and Contamination of Over-the-Counter Weight-Loss Products. AAPS PharmSciTech. 2021;22:78. Available from: https://link.springer.com/10.1208/s12249-021-01946-7.
30. Coghlan ML, Maker G, Crighton E, Haile J, Murray DC, White NE, et al. Combined DNA, toxicological and heavy metal analyses provides an auditing toolkit to improve pharmacovigilance of traditional Chinese medicine (TCM). Sci Rep. 2015;5:17475. Available from: http://www.nature.com/articles/srep17475.
31. Yilmaz KB, Sabuncuoglu B, Yildirim B, Silberschmidt VV. A brief review on the mechanical behavior of nonwoven fabrics. J Eng Fibers Fabr. 2020;15:155892502097019. Available from: http://journals.sagepub.com/doi/10.1177/1558925020970197.
32. Choi SM, Song JK, Kim SJ. Determination of Heavy metals on the non-woven in wet wipes using ICP-MS. J Oil Applied Sci. 2016;33:195-203. Available from: https://doi.org/10.12925/JKOCS.2016.33.1.195.
33. European Parliament. Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use. 2001.
34. Bouvier G, Boiteau JG, Gras A, Luzt AP, Etchegaray JP. Determination of Dermal PDE for Pharmaceutical Products. Pharmac Technology. 2018;42:38-40.
35. Rodler S. Elemental impurities in drug products: Challenges for the pharmaceutical industry in establishing limits based on ICH Q3D for alternative routes of administration (Master of Drug regulatory Affairs). Bonn; 2019.
36. Chaparro Leal LT, Guney M, Zagury GJ. In vitro dermal bioaccessibility of selected metals in contaminated soil and mine tailings and human health risk characterization. Chemosphere. 2018;197:42-9. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0045653518300080.
37. Lim DS, Roh TH, Kim MK, Kwon YC, Choi SM, Kwack SJ, et al. Non-cancer, cancer, and dermal sensitization risk assessment of heavy metals in cosmetics. J Toxicol Environ Health A. 2018;81:432-52. Available from: www.tandfonline.com/doi/full/10.1080/15287394.2018.1451191.
38. Ahlström MG, Thyssen JP, Wennervaldt M, Menné T, Johansen JD. Nickel allergy and allergic contact dermatitis: A clinical review of immunology, epidemiology, exposure, and treatment. Contact Dermatitis. 2019;81:227-41. Available from: https://onlinelibrary.wiley.com/doi/10.1111/cod.13327.
39. Hagvall L, Pour MD, Feng J, Karma M, Hedberg Y, Malmberg P. Skin permeation of nickel, cobalt and chromium salts in ex vivo human skin, visualized using mass spectrometry imaging. Toxicol in Vitro. 2021;76:105232. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0887233321001570.
40. Kimber I, Gerberick GF, Basketter DA. Thresholds in contact sensitization: Theoretical and practical considerations. Food Chem Toxicol. 1999;37:553-60. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0278691599000484.
41. Marinovich M, Boraso MS, Testai E, Galli CL. Metals in cosmetics: An a posteriori safety evaluation. Regul Toxicol Pharmacol. 2014;69:416-24. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0273230014000877.