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Original article

Quality of dorzolamide hydrochloride and timolol maleate containing eye drops distributed online

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ABSTRACT

Patient safety risks associated with the online purchase of medications, especially in case of ophthalmic preparations, are significant. Our study aimed to carry out quality assessment of dorzolamide hydrochloride (DZA) and timolol maleate (TIM) eye drops preserved with benzalkonium chloride (BAC) via online test purchases.

Three samples were purchased online, while control preparations were acquired through authorized national drug supply chain. Our method was based on the International Pharmaceutical Federation (FIP) Inspection Checklist and integrated the evaluation of packaging and labelling. Sterility was established according to the European Pharmacopoeia (Ph. Eur.), while qualitative and quantitative quality was assessed with high-performance liquid chromatographic (HPLC) analysis.

Several signs of falsification were recognized upon visual inspection of the online samples. All the products were clear, colourless, slightly viscous solutions. They were free from visible contaminants. The samples were sterile as no evidence of microbial growth was found. A quick and inexpensive HPLC analysis, optimized by the authors showed that active ingredients and the preservative deviated significantly ($p < 0,05$) with more than 10% from the values stated on the labels for at least one component (DZA: 99.3–113.1%, TIM: 112.8–139.2%, BAC: 82.4–97.7%).

Development of comprehensive and reliable quality assessment methods are vital to increase public safety of pharmaceutical products sold online. A complex approach, integrating visual inspection, labelling assessment, microbiological analysis coupled with qualitative and quantitative methods provide a most reliable method. Due to its limited feasibility and cost-effectiveness, raising public awareness and limiting illegal online sellers should be the primary approaches to protect patients from substandard and falsified medicinal products sold via the internet. Particularly important for health professionals to

Abbreviations: BAC, benzalkonium chloride; DZA, dorzolamide hydrochloride; EMA, European Medicines Agency; FIP, International Pharmaceutical Federation; ICH, International Council on Harmonisation; Laboratory of the local hospital, the Medicopus laboratory of the Somogy County Kaposi Mór Teaching Hospital, Kaposvár, Hungary; National microbiological laboratory, the Pharmavalid Ltd, Budapest, Hungary (GMP OGYEI 26495–5/2019); Our country, Hungary; Ph.Eur., European Pharmacopoeia; RSD, relative standard deviation; SF, substandard and falsified (medical product); TIM, timolol maleate; WHO, World Health Organization.

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understand this market and its public health concern, and to raise patient awareness of the risks associated with uncontrolled online purchase of medication.

1. Introduction

The internet is a popular place to purchase products and services, including medications. Approximately 60% of internet users buy health-related products in Japan and the USA (Khan et al., 2012). According to the representative direct questionnaire survey of Fittler et al. the respondents (n = 1055) already bought medicines (4.17 %) and other health products (18.4 %) via the internet (Fittler et al., 2018a). A significant increase in the frequency of buying medicines (55.48%) and health products (63.0%) online was measured in a recent online survey following the coronavirus pandemic (Fittler et al., 2022). There are numerous patient safety risks associated with obtaining medicines outside the official supply chains. These are misleading product information, rogue online pharmacies and counterfeit medications. The global market of counterfeit medicines is a public health concern which affects nearly every country in the world. The origins of the substandard and falsified (SF) medical products are not the same in the developing and developed countries. The main source is the internet (WHO, 2017; Vida et al., 2017; Fittler et al., 2018b) in developed countries. With the increasing online trade more and more low-quality pharmaceutical products enter into circulation (Rahman et al., 2021; Ho et al., 2022). Most of the studies from this field analysed the online availability and quality of medications or other healthcare products procured via the internet (Orizio et al., 2011; Norbutas, 2018; Vida et al., 2017, 2019, 2020).

In our previous analysis we reviewed patient safety of online purchased medications (Vida et al., 2020). We aimed to develop a tool for identifying high risk medicinal products sold on the internet. We developed a complex risk assessment method which entails the general pharmaceutical risk, the therapeutic risk, the risk originated from the likelihood of microbiological contamination, the risk originated from the limited access to the product and the risk of counterfeiting the medication. We directly focused on high-risk products, ophthalmic preparations as models to illustrate the real-world applicability of the tool. In relation to these medications, not only the illegal online source or access without prescription, but also undesirable quality (especially microbiological contamination), and the inappropriate use or misuse may cause serious health damage (Mayo et al., 1987; Snyder and Glasser, 1994; Donzis, 1997; Morales et al., 2016; Vaajanen and Vapaatalo, 2017; Gao et al., 2018).

Based on the results of our previous analysis we selected dorzolamide hydrochloride (DZA) and timolol maleate (TIM) combination eye drops for test purchase (Vida et al., 2020). This product contains these active ingredients in fixed ratio and has been developed to treat open-angle glaucoma (Inoue et al., 2012). These prescription-only products have been available in our country since 2000. The European consumption of DZA and TIM combined eye drops was about 6.5 million packs in the last 5 years. Our national market share from this amount was 2.11 % (IQVIA™, 2023). Currently 8 preparations are on the market. Most of them (6) contain benzalkonium chloride (BAC) as a preservative, while two of them are preservative-free. The preservative free products use a special filter or unit dose packaging method to maintain microbiological stability (NIPN, 2020). Apart from the active ingredients (20 mg/ml dorzolamide in 22.26 mg/ml DZA and 5 mg/ml timolol in 6.83 mg/ml TIM) the eye drops contain the preservative BAC (0.075 mg/ml) and hydroxyethyl cellulose, mannitol, sodium citrate, sodium hydroxide and water.

We test purchased 3 multi-dose DZA and TIM combination eye drops with preservative BAC over the internet between November 2018 and January 2019. The safety risks are systemic side effects, inappropriate use, microbial contamination, uncontrolled free availability, and substandard ingredients. The potential patient harm originating from using substandard or falsified products can only be determined by chemical analysis and electronic verification (if applicable).

Our objective was to assess the pharmaceutical quality of online purchased multi-dose eye drops containing DZA and TIM preserved with BAC.

2. Material and methods

The methods of the assessment used in this study followed the guidelines of the World Health Organization (WHO) and methods outlined in various research papers (Newton et al., 2009; Rahman et al., 2021; Veronin et al., 2014; Vida et al., 2017; World Health Organization (WHO), 2016; World Health Organization WHO, 1999).

2.1. Sample collection

Based on the methodology of Vida et al. (Vida et al., 2017) we identified online distributors of eye drops using the search terms "*buy dorzolamide/timolol eye drops*" in Google and Yahoo search engines between November 2018 and January 2019. We evaluated the first 150 pages of online research of which 29 were websites, and 26 were online pharmacies. Our selection criteria were possible patient and medication safety concerns: website characteristics, legality (LegitScript, 2016), popularity (Offer, 2007), storage conditions, delivery options and time, packaging and attached product information leaflet. We found only five potential illegal online pharmacies which sold the searched multi-dose eye drops without prescription. Three orders out of five arrived. There was only one occasion where prescription was required.

The following pharmaceutical formulations were purchased from online pharmacies according to our pre-purchase risk assessment method (Vida et al., 2020). Online Sample No.1 and No.3 were Dorzox T eye drops (on the label contains DZA, United States Pharmacopeia (USP) equivalent to dorzolamide 2% w/v + TIM, IP, equivalent to timolol 0.5% w/v; Cipla LTD, Mumbai, India). We purchased them from Trusted Tablets (https://www.trustedallovetheworld.com) (Online Sample No.1) and Buy Pharma (https://www.buy-pharma.md) (Online Sample No.3) respectively. Online Sample No.2 was Cosopt eye drop (on the label contains DZA, USP equivalent to dorzolamide 20 mg/ml + TIM, IP, equivalent to timolol 5 mg/ml; Santen OY, Japan). We purchased it from GuysPharmacy (https://www.kiwidrug.com). Cosopt eye drop (labelled to contain DZA, USP equivalent to dorzolamide 20 mg/ml + TIM, IP, equivalent to timolol 5 mg/ml; Santen OY, Japan) was purchased from the official national drug supply chain was used as Control Sample. Online Samples No.1 and No.3 were available without prescription, while Online Sample No. 2 and the Control Sample required prescription.

2.2. Sample analysis

Quality assessment of the eye drops were carried out according to WHO protocols (WHO, 2016). We stored the samples in air-conditioned laboratory (20–25 °C). Photos of the outside and inside leaflets and labels were taken before analysis. We conducted physical, chemical and microbiological analyses of all samples before the stated expiry date.

The performed analytical tests of the eye drops detected the presence and the concentration of the DZA, TIM and BAC of the product. Almost all of samples (5.0 ml) were used for sterility testing. Therefore, in our investigation we first set up the HPLC method and tested the eye drops because of the small quantities of the online purchased samples. We took samples for analysis with small sampling isolation method in a TEMA Flex GMP grad A, aseptic isolator, with laminar flow. The isolated samples were placed in 0.7 diameter, 1 ml colourless glass test tubes with PE closures. Physical and chemical assessment of the quality of eye drops was carried out at the laboratory of the local hospital. The microbiological assay of the products was performed by a national microbiological laboratory.

The analyses consisted of a visual inspection and general quality analysis of the dosage form including visual check, identification and specificity assay and sterility testing (Aldrich et al., 2013).

2.2.1. Visual inspection of test purchase samples

The visual inspection involved observation and description of the primary and secondary packaging conditions (the container and the carton protecting the container), leaflets and labelling of the eye drops. This was carried out according to the FIP Inspection Checklist (FIP, 2013). We slightly adjusted them for the drug dosage forms. We also evaluated the indicators of substandard or falsified medicines, such as the container, the closure, the labels and the leaflet.

2.2.2. Visual check of the sample solution

The specification of the manufacturer and the Control Sample's summary of product characteristics claims that the sample solutions must be clear, colourless to almost colourless, slightly viscous, free from contaminants by organoleptic tests (CiplaMed, 2018; NIPN, 2021). The purity and the colour of our samples were compared to the similar amount of the control sample in the small test tubes. We performed the comparison visually in diffused daylight, in front of a white background following Ph. Eur. as guideline (Ph. Eur., 2008).

2.2.3. Identification and specificity assay with HPLC

Several analytical methods for detecting DZA, TIM and BAC were published in scientific reports and in the pharmacopoeias (EU, British and USP). We adapted and applied, optimized and validated with the help of the tools at our disposal, rapid, simple, cost-effective and repeatable HPLC methods (AlAani and AlNukkary, 2016; Baker and Belal, 2018; Erk, 2002, 2003; Ibrahim, 2019; Liu et al., 2009; Mehta et al., 2010; Mohamed et al., 2014; Nagori et al., 2011; Rojsitthisak et al., 2005; Shadoul et al., 2011; Thangabalan et al., 2018; Wanare et al., 2012; Chengalva et al., 2016).

As a result of the analytical method optimization the isocratic HPLC methods were executed on Waters SPHERISORB ODS1 C18 (5 μm, 250 mm × 4.6 mm) column at room temperature, in acetonitrile–phosphate buffers (pH 2.50, 20:80 v/v) (DZA, TIM) (Eluent B), (pH 5.65, 70:30 v/v) (BAC) (Eluent BAC). The eluent flow rate was 1.0 ml/min, the UV detections were performed at 210 nm (BAC), 250 nm (DZA) and 300 nm (TIM) at room temperature. The methods are simple, quick, and inexpensive because the components can be measured without regenerating the system.

We validated our HPLC method to conform to the international guidelines given by the International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) of the European Medicines Agency (EMA) (EMA, 1995). The standard solutions of the studied drugs were prepared with HPLC water (DZA 800 μg/mL, TIM 1000 μg/mL, BAC 150 μg/mL). They were diluted with HPLC water to prepare working solutions containing 25–800 μg/mL, 50–1000 μg/mL and 10–150 μg/mL DZA, TIM and BAC, respectively. The dilution series were analysed in three replicates under optimum chromatographic conditions. Calibration curves and regression equations were obtained by plotting the mean peak areas of each drug against the corresponding concentrations.

We used unpaired *t*-test to calculate the significant differences in 95% confidence interval.

2.2.4. Sterility testing in compliance with the European Pharmacopoeia

For sterility testing the membrane filtration technique was used to ensure sterility in compliance with the Ph. Eur. (Ph. Eur., 2009). Samples were filtered on a mixed cellulose ester membrane filter with a pore size of 0.45 μm and 50 mm diameter (Sartorius, Sigma-Aldrich, Steinheim, Germany). For detecting anaerobic bacteria, we used thioglycollate liquid culture medium (Thio). For detecting fungi and aerobic bacteria we used soya-bean casein digest medium (CASO) which was manufactured by the laboratory. A sterile 1 g/l casein peptone solution (pH 7.1) was used for dilution and washing (2 X 100 ml). The Thio medium was incubated at 32° C for 3 days, the CASO medium was incubated at 23° C for 3 days (for bacterium detection) and 5 days (for fungus detection). When no microbial growth was detected, the products were regarded sterile. The validation of this sterility assay was also performed with the Control Sample (6 X 10 ml) in compliance with the Ph. Eur. The test microorganisms were *Staphylococcus aureus* (ATCC 6538, 24 CFU/0.1 ml), *Bacillus subtilis* (ATCC 6633, 21 CFU/0.1 ml), *Pseudomonas aeruginosa* (ATCC 9027, 91 CFU/0.1 ml), *Clostridium sporogenes* (ATCC 19404, 69 CFU/0.1 ml), *Candida albicans* (ATCC 10231, 79 CFU/0.1 ml), *Aspergillus brasiliensis* (ATCC 16404, 24 CFU/0.1 ml).

2.3. Content compliance criteria

Eye drops must be sterile and free from physical, chemical and biological contaminants (Ph. Eur., 2009; Uddin et al., 2017), and must contain 90.0–110.0% the amount of active pharmaceutical ingredients stated on the labels of their containers (Sharath et al., 2011; Kahook et al., 2012; Tatham, 2020).

3. Results

3.1. Visual inspection

The visual inspections of medicines predict their substandard quality and falsification (eg improper packaging and labelling). We checked the packaging and compared them with the containers used by original manufacturer. We also checked the documents came with the product. We did not consider the colour change of the logo or hologram listed in the FIP inspection checklist as it was not available in our study. Further, we did not consider the first two questions in section "2.8. Dosage statement" since the Control Sample also lacked this information. Finally, we used 34 check points of the 37 on the FIP list. Online Samples No. 1 and No. 3 were non-compliant in 8 check points (24 %), while Online Sample No. 2 was non-compliant in 6 points (18 %). We observed several potential signs of falsification on the label during

Fig. 1. Package and container of Online Sample No. 1.

Fig. 3. Package and container of Online Sample No. 3.

inspection. These were the trade name, the manufacturer, the strength of the medicine, the dosage form and the serialization. The three Online Sample eye drops are illustrated in Figs. 1-3**.**

The results of the visual inspections according to the inspection checklist of the FIP are summarised in Table 1.

3.2. Visual check of the sample solution

Organoleptic inspection the physical appearance of the samples was found to be compliant with the Ph. Eur. standards. All online samples were clear, colourless, slightly viscous solutions and were free from visible contaminants.

3.3. Identification and specificity assay with HPLC

The retention times for DZA and TIM were at 4.40 and 7.20 min. The BAC had three homologues and resulted in peaks at 5.90 min, 8.89 min and 16.15 min.

The methods were validated in full accordance with EMA ICH guidelines and the Ph. Eur. The results of system suitability parameters were within the acceptable limits of the EMA guidelines (Table 2). This demonstrated the suitability, linearity, accuracy, precision, sensitivity, stability and robustness of this analytical method.

The samples were tested following 10-fold (TIM and BAC) and 40-fold dilutions (DZA) because of the sensitivity of the detector. The dilutions were prepared with HPLC grade water in three replicates and eluted under the optimum chromatographic conditions. The nominal concentration of each drug (Cmeasured) was calculated from the regression equation (Table 3).

All online procured eye drops contained the active ingredients (DZA, TIM) and the preservative (BAC). As shown in Table 3, the concentrations ($\mathbf{C_{measured\ mean}}$) of the online samples, for at least one component differed significantly from the values measured in the Control Sample with more than 10% ($p < 0.05$). The online purchased eye drops contained 99.3–113.1% DZA, 112.8–139.2% TIM and 82.4–97.7% BAC compared to the quantities stated on the labels. They also contained 87.0–99.2% DZA, 91.7–113.2% TIM and 88.9–105.5% BAC in compared to the values measured in the Control Sample.

3.4. Sterility

No evidence of microbial growth was found, so the online samples No. 1, No. 2 and No. 3 were considered sterile.

4. Discussion

Thorough visual inspection is essential to identify substandard and falsified medicinal products. All online samples failed the inspection checklist of FIP due to several reasons, all indicating potential patient safety concerns. Origin and appropriate use of

Fig. 2. Re-labelled package and re-labelled container of Online Sample No. 2.

Table 1
Visual inspection of the eye drops based according to FIP checklist.

Inspection categories of packaging and labelling	**Specific questions of the visual inspection** (applicable responses: Yes / No / UNK. / N/A)	Control Sample	Online Sample No. 1	Online Sample No. 2	Online Sample No. 3
1. Container and Closure	Does the container and closure protect the product from the outside environment, eg. is the container properly sealed?	YES	YES	YES	YES
	Do they assure that the product will meet the proper specifications throughout its shelf life?	YES	YES	YES	YES
	Are the container and the closure appropriate for the product inside? (according to the leaflet)	YES	YES	YES	YES
	Is the container safely sealed?	YES	YES	YES	YES
2. Label	If there is a carton protecting the container, does the label on the carton match the label on the container?	YES	YES	NO[a]	YES
	Is all information on the label legible and indelible?	YES	YES	NO[b]	YES
2.1. The trade (brand) name	Is the trade name spelled correctly?	YES	YES	YES	YES
	Is the medicinal product (trade name) registered in the country by the Drug Regulatory Authority? So is the product legally sold in the country?	YES[c]	NO[d]	YES[c]	NO[d]
	Does the symbol ® follow the trade name?	YES[c]	NO[d]	YES[c]	NO[d]
	Is the trade name indelibly impressed or imprinted onto the container?	YES[c]	YES[d]	NO[b]	YES[d]
2.2. The name of the active ingredient (scientific name/generic name)	Is the active ingredient name spelt correctly?	YES[e]	YES[e]	YES[e]	YES[e]
	Do the trade name and the active ingredient names correspond to the registered product? [f]	YES[e]	YES[e]	YES[e]	YES[e]
2.3. The manufacturer's name and logo	Are the manufacturer's name and logo legible, and correct?	YES[g]	YES[h]	YES[g]	YES[h]
	Does the logo or hologram (if applicable) look authentic?	YES[g]	YES[h]	YES[g]	YES[h]
	Does the logo or hologram (if applicable) change colour when viewed from different angles?	N/A	N/A	N/A	N/A
2.4. The manufacturer's full address	Is the manufacturer's full address legible, and correct?	YES[i]	YES[j]	YES[k]	YES[j]
	Has this company or its agent registered the product in the country?	YES	NO	YES	NO
2.5. The medicine strength	Is the strength - the amount of active ingredient per unit - clearly stated on the label?	YES	YES	YES	YES
	For container packed products, is the medicine strength indelibly impressed or imprinted onto the container?	YES[c]	YES[d]	NO[b]	YES[d]
2.6. The dosage form	Is the dosage form clearly indicated on the container label?	YES	YES	YES	YES
	Does the dosage form stated on the label match the actual dosage form of the medication?	YES	YES	YES	YES
	Is the indicated medicine under this dosage form registered and authorized for sale in the country?	YES	NO	YES	NO
2.7. The number of units per container	Does the number of dosage units listed on the label match the number of dosage units stated on the container?	YES	YES	YES	YES
2.8. Dosage statement (if appropriate)	Is the dosage clearly indicated on the label?	NO	NO	NO	NO
	Is the dosage stated on the label appropriate for the medicine in this form and strength?	NO	NO	NO	NO
	Is the product registered and authorized for sale in the country with this dosage?	YES	NO	YES	NO
2.9. The batch (or lot) number	Does the numbering system on the package correspond to that of the producing company?	YES	N/A	YES	N/A
	For container packed medicines, is the batch number indelibly impressed or imprinted onto the label of the container?	YES	YES	YES	YES
2.10. The date of manufacture and the expiry date	Are the manufacture and expiry dates clearly indicated on the label?	YES	YES	YES	YES
	For container packed products, is the expiry date indelibly impressed or imprinted onto the label of the container?	YES	YES	YES	YES
2.11. Storage information	Are the storage conditions indicated on the label?	YES	YES	YES[b]	YES
	Has the product been properly stored?	YES	UNK.[l]	UNK.[l]	UNK.[l]
2.12. Safety features and serialization	Is the unique identifier (datamatrix) code available on the outer package?	YES	NO	NO	NO
	Is an anti-tampering device available on the packaging?	YES	YES	YES	YES
3. Leaflet or package insert	Is the package insert printed on the same colored or same quality paper as the original (If available to compare) or does it look familiar?	YES	YES	YES[m]	YES
	Is the ink on the package insert or packaging smudge-proof?	YES	YES	YES	YES
	Does the information on the package insert match the information on the product container?	YES	YES	YES	YES

N/A = not available.
[a] Cosopt® relabeled by English subtitle, but the Italian subtitle is also visible on the carton but not on the container.
[b] Cosopt® relabeled by a removable English subtitle.
[c] Cosopt®.
[d] Dorzox T.
[e] DZA/TIM.
[f] According to the country of registration.
[g] Santen.
[h] Cipla.
[i] Santen Oy Finland.
[j] Cipla India.
[k] Santen Italy.
[l] UNK. = unknown.
[m] English, but the outsider package originally Italian.

Table 2
System suitability test results.

Parameter	Standard value	DZA	TIM	BAC homologues	
Mobile phase		Eluent B	Eluent B	Eluent BAC	
Wavelength (nm)		250	300	210	
Calibration range (μg/ml)		25.0–800.0	55.6–1011.1	9.47–142.0	
Correlation coefficient (R^2)	> 0,990	0.997	0.999	0.991	
Detection limits (μg/ml)		0.013	0.003	0.005	
Quantitation limits (μg/ml)		0.043	0.009	0.017	
RSD% of area	≤ 20 %, for n = 6	0.789	1.618	3.921	4.678
RSD% of retention time	≤ 1 %, for n = 6	0.108	0.136	0.208	0.278
Tailing factor (T)	≤ 2	1.72	1.31	1.89	1.56
Resolution (R)	> 2	–	–	7.66	

Table 3
The concentrations of the active ingredients and the preservative of the investigated eye drops.

		$C_{labelled}$ (mg/ml)	$C_{measured\ mean}$±SD (mg/ml)	p-value	Mean ($C_{measured}$/ $C_{labelled}$)±SD (%)	Mean ($C_{measured}$/ $C_{control}$)±SD (%)
Online Sample No.1	DZA	20.00	19.85 ± 0.07	0.0005	99.3 ± 0.33	87.0 ± 0.29
	TIM	5.00	6.96 ± 0.08	0.0174	139.2 ± 1.57	113.2 ± 1.27
	BAC	0.075	0.069 ± 0.0027	0.7829	92.0 ± 3.62	99.3 ± 3.91
Online Sample No.2	DZA	20.00	22.63 ± 0.27	0.6133	113.1 ± 1.36	99.2 ± 1.19
	TIM	5.00	6.15 ± 0.032	0.9936	123.0 ± 0.64	100.0 ± 0.52
	BAC	0.075	0.062 ± 0.0027	0.0104	82.4 ± 3.59	88.9 ± 3.88
Online Sample No.3	DZA	20.00	20.22 ± 0.58	0.0043	101.1 ± 2.90	88.7 ± 0.55
	TIM	5.00	5.64 ± 0.25	0.1094	112.8 ± 5.00	91.7 ± 4.07
	BAC	0.075	0.073 ± 0.0034	0.1405	97.7 ± 4.53	105.5 ± 4.89
Control Sample	DZA	20.00	22.81 ± 0.50	reference	114.0 ± 2,52	100.0 ± 0.00
	TIM	5.00	6.15 ± 0.35	reference	123.0 ± 7.02	100.0 ± 0.00
	BAC	0.075	0.069 ± 0.0012	reference	92.6 ± 1.54	100.0 ± 0.00

Online Sample No. 2 is questionable as it was relabelled from Italian to English. The labels on the package and the container were incongruent and were not fully legible. The manufacturers of counterfeit products pay less attention to the small details of the packaging and labelling, because they require a complex and an expensive manufacturing process (FIP, 2013). The lack of national authorisation of a medicinal product in the country of destination may not allow patients to find reliable product information and manufacturing conditions. Online Samples No. 1 and No. 3 were unregistered and unauthorized in our country. The delivery of such medications is a potential sign of falsification.

The analyses of the eye drops purchased in online markets identified minor discrepancies between the labelled and measured concentrations of active and preservative ingredients. The active ingredients of the Online Samples No. 1 and No. 3 did not meet the internationally accepted ± 10% threshold limit ($p < 0.05$). The Online Sample No. 2 also fell short of these standards because the preservative (BAC) was only 82% of the stated value ($p < 0.05$). The reduced active ingredient content may be due to inappropriate shipping and storage conditions, although all the components may be stored at room temperature. Admittedly, the eye drops obtained from the official national supply chain were within the allowed quantity range. An often-neglected property of test purchases for online medicines is sterility, an attribute with high priority for ophthalmic preparations. All samples were sterile in our test purchases. This parameter is of key importance during the manufacturing process, and the main aspect of quality and patient safety of the eye drops. Although all evaluated samples were free from microbiological contamination, sterility is not sufficient for the safety of the product. Proper storage and packaging are also essential for the stability of the ingredients.

Counterfeit medicinal products are difficult to be identified because they are nearly identical to the genuine medicines. The packaging and labelling indicated non-compliance, but the sterility tests did not reveal microbiological contamination.

Our findings proved that uncontrolled online drug purchases, especially in the case of prescription and high patient safety risk drugs, could cause health damage.

The major strength of our study is that we used samples from test purchases with predefined risk assessment. Our combination of a simple visual inspection test, a complex, rapid and cost-effective HPLC test and a pharmacopeial sterility test created a comprehensive evaluation method for medicinal products purchased on the internet. Further, the applied HPLC analysis of DZA, TIM and BAC and the method of sterility are accurate and suitable for routine quality assurance of small samples for further studies. The limitations of our work are the relatively small sample sizes and the time-consuming nature of the combined methodology, limiting its on-site application and generalizability for online medicine quality assessment. We would like to further develop and amend our techniques to be able to implement rapid and mobile quality assessments of medicinal products purchased via the internet.

5. Conclusions

Internet purchases of medications are a global phenomenon which is growing and affecting every health care system. Online buyers are often unaware that these products do not undergo the quality control that is always required for authorized medicines. Therefore, they may not be aware of the quality issues associate with products purchased via the internet. Authentic and evidence-based information from health professionals may help increase health awareness and reduce individual risk. Regular assessment of content and labels of medicinal products sold on the internet are essential to increase patient safety. Easy-to-implement tests are required to filter out counterfeit and falsified medicines. These assessments may help formulate new international regulations, awareness campaigns and legal enforcements.

It is therefore particularly important for health professionals to understand this market, and to raise patient awareness of the risks associated with uncontrolled online purchase of medication. They must be given accurate information on these products, so that they can rapidly identify the side effects and harms these products may cause.

CRediT authorship contribution statement

Sára Merczel: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Writing – original draft, Visualization, Project administration. **Róbert György Vida:** Conceptualization, Methodology, Writing – review & editing. **Tamás Tasi:** Methodology, Software, Validation, Formal analysis, Investigation, Resources, Writing – review & editing. **András Fittler:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, Funding acquisition. **Lajos Botz:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethics approval and consent of participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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