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Short communication

Analysis of Purity Profiles of Generic Lisinopril Tablets Marketed in Croatia

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A B S T R A C T

In view of an increasing number of generic drugs emerging, a comparative study was performed including the approved lisinopril preparations in the form of tablets marketed in Croatia, to compare purity profiles of generic drugs versus the original medicinal product. Several batches of each individual medicinal product at different stages of their shelf life were analyzed. Impurities were determined by means of high performance liquid chromatography (HPLC). Impurity profiles were demonstrated to be specific for each individual drug. Original drug, as compared to its generic copies, had the lowest values and also the lowest variability of all the tested parameters – type, total number and content of impurities – suggesting that its manufacturing process is to certain degree better controlled compared to other manufacturers. A characteristic impurity C appearing in all the assessed preparations has the lowest levels in the original drug, whereas the amount of the highest unknown impurity does not exceed 0.10% in any of the analyzed preparations. Although the original drug stands out from all the generic preparations with its purity, it can be generally concluded that, as regarding impurities levels, all the analyzed medicinal products are within the ranges of specification limits; accordingly, it is therefore not expected that, in case of lisinopril tablets, administration of the original drug as compared to any of its generic drugs, will be safer for the patient.

Key words*: generic drug, original drug, quality control, drug impurity, comparative study, HPLC, drug safety, drug shelf life*

Introduction

The safety of drug administration is one of the major pillars supporting modern pharmacy and medicine with the issue of efficient control over drug safety being of the increasing importance for the work of regulatory bodies in that area[1]. Therapy safety is primarily determined by undesirable effects (side effects) of the drug resulting mainly from the properties of the active substance itself. However, impurities may also be the cause of drug undesirable effect and it is actually for this reason that the effective control impurities in medicinal products contributes to a large extent to the safety of their administration[2–4]. From the historical point of view, it is in the area of purity that the largest changes in drug quality control occurred, where improvements in technology and parallel development of new and/or advanced existing methodologies enabled a more detailed insight into the presence of impurities in medicinal products, their isolation, identification and quantitative determination[2,4,5].

The most represented type of impurities contained in medicinal products is organic impurities with chemical structure similar to the active substance. Such impurities are the largest source of danger for potential contribution to an undesirable effect of the drug due to their mere presence or to being present in certain amount causing the undesirable effect[2]. Almost all the impurities of organic origin can be identified applying chromatographic or related techniques, with the high performance liquid chromatography (HPLC) being the dominant analytical technique among them[6].

The therapy for a chronic disease implies daily intake of a drug into the organism for a longer period, with the issue of safe administration of such a drug additionally gaining in importance. The parameter of purity of such drugs as a key parameter of quality that is essential for the safety of their administration requires closer con-

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trol[7]. This is supported by the results of a study conducted by Y. Gomez et al.[8] who compared the quality of medicinal products of clopidogrel tablets. In the study, eighteen generic copies were compared to the original drug according to the following quality parameters: uniformity of mass, impurity profile, content, dissolution properties and stability. It was found out that the majority of generic drugs were not of a comparable quality as related to the original drug, because they contained a larger amount of impurities and also due to detection of significant differences relating to other tested parameters.

Realizing that certain markets in the world supply medicinal products of the quality not comparable to that of the reference drug, and in the context of the increasing number of generic drugs emerging both globally and on the market of Croatia, a target study was conducted on impurity profiles of drug product tablets containing lisinopril that have been approved and sampled from the market with the purpose of comparing the purity of generic preparations in relation to the original drug. Angiotensin-converting enzyme (ACE) inhibitor lisinopril was selected as the representative of the increasing group of drugs sharing the common property of being introduced into the organism on a daily basis for a longer period and that are marketed in Croatia in a large and increasing number of generic preparations. There were 10 preparations of lisinopril tablets approved in Croatia at the time of the study (i.e. from April 2009 until January 2010) in comparison to only 1 preparation approved in 1999. The study aimed at encompassing as broadly as possible the spectrum of lisinopril preparations marketed in Croatia and at obtaining an insight into, let's say, »purity state« of the drug at a certain point of the anticipated shelf life. For this reason, target analyses were carried out of several batches of each individual drug (depending on the extent of its representation on the market in the course of the study) and specifically at a different stage of its shelf life. To collect a desired number of samples, the study was conducted for 10 months.

Determination of impurities was performed applying the method of liquid chromatography as described in the European Pharmacopoeia[9] with certain modifications as described in the section »Materials and Methods«. The following parameters were observed and served as the basis for a comparison of drugs: impurity profile, amount of total impurities and the total number of detected impurities.

Materials and Methods

Chemicals

Lisinopril dihydrate for performance test CRS (chemical reference substance, according to the nomenclature of reference substances in European Pharmacopoeia (Ph. Eur.) established by the European Directorate for the Quality of Medicines & HealthCare (EDQM)) was purchased from the European Directorate for the Quality of Medicines & HealthCare (EDQM) – Council of Europe, Strasbourg, France. Acetonitrile of HPLC purity grade was purchased from Merck KGaA, Darmstadt, Germany, sodium dihydrogen phosphate p.a. and sodium hydroxide p.a. from Kemika d.d., Zagreb, Croatia. Membrane filters of 0.45 μm (Spartan 30/0.45 RC) were bought from Whatman GmbH, Dassel, Germany. Employed in the assays was »in-house« purified water.

Chromatography

Chromatographic analyses were carried out on the Agilent 1200 RR Series HPLC System. For the separation of impurities, as stationary phase, a Zorbax Eclipse XDB C18 column (4.6 mm x 100 mm, 3.5 μm) thermostated to 50 °C was used. Gradient elution was performed with the mixture of buffer and acetonitrile at the ratio of 97:3 (V/V) in the channel A, and with the mixture of buffer and acetonitrile at the ratio of 80:20 (V/V) in the channel B. Gradient elution developed according to the following scheme: isocratic elution from the channel A up to 2 minutes, linear gradient of 0% B in the 2nd minute up to 55% B in the 20th minute, 0% B in the 21st minute and postrun 9 minutes. The buffer was prepared by dissolving 26 mmol/L of sodium dihydrogen phosphate in purified water, and the pH of the solution was then adjusted with NaOH to pH 5.0. The flow rate was set to 1.0 mL/min with 10 μL injection volume while detection was performed at 210 nm.

Sampling

The samples of the lisinopril tablets were collected from the Croatian market. In choosing samples, the principal criterion was the shelf life, aiming to obtain distribution of different batches of the individual drug in the course of its shelf life. Since the approved shelf life differs from drug to drug, it was not possible to take the shelf life alone as the measure for comparison of drug »age«. For that purpose, an index of the shelf life (IRV) was introduced and defined by the ratio of time remaining from the day of analysis until the expiry date (V) and the time of the approved (theoretical) shelf life (RV):

$$IRV = 1 - \frac{V(days)}{RV(days)} .$$

The range of IRV values the analyzed drug can have is from 0 to 1. The drug with a higher IRV value is at a late stage of its shelf life i.e. has less time remaining until its expiry date. Since not all the drug are equally represented on the market, endeavour was made, for the duration of the study, to cover the largest possible number of stages within the approved shelf life for each individual drug or, in other words, the aim was to collect as many different batches as possible of the individual drug exhibiting different IRV values. Thus, four products were analyzed at five or more different stages of the shelf life (a different IRV value rounded up to one decimal place), two products at four and one of each product at three, two or one stage of the shelf life respectively (Table 1).

Commercial names and manufacturers of medicinal products included in this study are specified in Table 2.

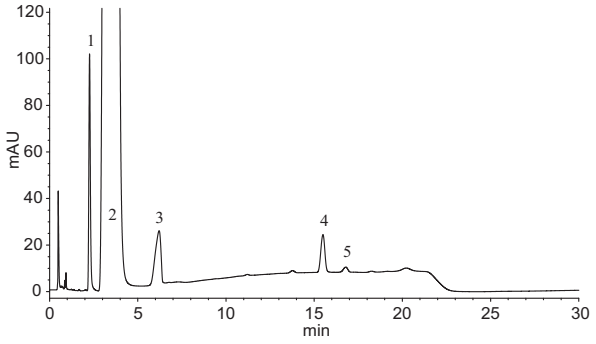

Fig. 1. Chromatogram of lisinopril dihydrate for performance test CRS. Peaks marked: 1 – impurity A, 2 – lisinopril, 3 – impurity E, 4 – impurity D, 5 – impurity C.

TABLE 1
ANALYZED SAMPLES

Total number of the registered drugs (lisinopril tablets): 10	Number of analyzed samples
At least 1 IRV	9
5 or more different IRV	4
4 different IRV	2
3 different IRV	1
2 different IRV	1
1 IRV	1
Not analyzed	1

IRV – index of the shelf life (explained in the section »Sampling«).

Sample preparation

5–10 tablets were crushed in a mortar and the quantity of powder equivalent to 25 mg of lisinopril (concentration of 1 mg/mL) was weighed into a 25 mL volumetric flask, about 2/3 of the solvent (the mixture of buffer and acetonitrile at the ratio of 97:3 V/V) was added and placed in an ultrasonic bath for 10 min. After making up with the same solvent to the volume, a portion of the solution was filtered and then diluted to the concentration of 0.01 mg/mL of lisinopril with the same solvent. The sample solution at the concentration of 1 mg/mL lisinopril was used to determine impurities. Quantification was performed by means of the diluted sample solution at the concentration of 0.01 mg/mL lisinopril (1%).

Results and Discussion

The European Pharmacopoeia Monograph on lisinopril dihydrate defines the list of known specific organic impurities that may be present in the formulations of medicinal products either as starting materials, synthesis by-products, intermediates, reagents, ligands, catalysts or degradation products (impurities A to F)[9,10]. Also defined by the same monograph are the specification limits for the amount of known impurities in the active substance lisinopril: 0.3% for individual and 0.5% for total impurities (without impurity E)[9]. The presence of known impurities as specified in monograph of the European Pharmacopoeia as well as of unknown nonspecified impurities at the level of method detectability of 0.01% was monitored in the analyzed samples. Quantification of detected impurities was performed for all the found impurities either equal or exceeding the quantification limit of 0.03%. System suitability was checked according to the Monograph by injecting the lisinopril dihydrate for performance test CRS solution. As evident from Figure 1, peaks due to impurity A and impurity E fall on either side of the peak due to lisinopril with distinct resolution between both pairs ($R_{A,liz}$ = 2.1; $R_{liz,E}$ = 3.8).

TABLE 2
OVERVIEW OF SAMPLES INCLUDED IN THE STUDY AND RESULTS FOR AVERAGE CONTENT OF IMPURITIES

Sample number	Branded name	Drug manufacturer (country)	Average amount of total impurities (% m/m)	Average amount of impurity A (% m/m)	Average amount of impurity C (% m/m)	Average amount of impurity E (% m/m)	Average amount of unknown impurities (% m/m)
Ref. drug	Prinivil	Merck Sharp & Dohme B.V. (Holland)	0.04	<0.03	0.04	–	<0.03
1	Amicor	Jadran Galenski laboratorij d.d. (Croatia)	0.39	0.05	0.15	0.09	0.09
2	Irumed	Belupo d.d. (Croatia)	0.17	<0.03	0.05	0.11	<0.03
3	Laaven	Krka d.d. (Slovenia)	0.33	<0.03	0.16	0.11	0.04
4	Lizinopril Farmal	Farmal d.d. (Croatia)	0.24	<0.03	0.11	0.13	–
5	Lizinopril Lek	Lek d.d. (Slovenia)	0.23	<0.03	0.09	0.10	0.05
6	Optimon	Pliva d.d. (Croatia)	0.21	<0.03	0.13	0.07	–
7	Skopryl	Alkaloid AD (Macedonia)	0.31	0.07	0.08	0.11	0.07
8	Vitopril	Stada Arzneimittel AG (Germany)	0.38	0.07	0.07	0.10	0.14
9	Lisinolex	Galex d.d. (Slovenia)	n.a.*	n.a.*	n.a.*	n.a.*	n.a.*

*n.a. – not analyzed since the drug was not found on the market in the course of the study

TABLE 3
AMOUNT OF TOTAL IMPURITIES, IMPURITY C AND THE NUMBER OF IMPURITIES FOR EACH ANALYZED DRUG BATCH COMPRISED WITH THE STUDY

Branded name	Number of analyzed batches	IRV	Total impurities (% m/m)	Impurity C (% m/m)	Number of impurities
Prinivil	1	0.1	<0.03	<0.03	1
	2	0.2	<0.03	<0.03	1
	3	0.3	0.04	0.04	1
	4	0.4	0.04	0.04	1
	5	0.5	0.04	0.04	1
	6	0.7	0.06	0.06	1
	7	0.8 (0.76)	0.06	0.06	3
	8	0.8 (0.84)	0.07	0.07	2
Amicor	1	0.7	0.32	0.13	6
	2	0.8	0.29	0.12	6
	3	0.9	0.33	0.14	6
	4	1.0	0.62	0.19	8
Irumed	1	0.1	0.12	<0.03	7
	2	0.2 (0.20)	0.14	0.05	6
	3	0.2 (0.20)	0.20	0.05	5
	4	1.0	0.21	0.09	6
Laaven	1	0.5	0.16	0.16	2
	2	0.6	0.21	0.07	5
	3	0.7	0.49	0.23	4
	4	0.8	0.21	0.07	4
	5	0.9	0.57	0.30	4
Lizinopril Farmal	1	0.8	0.24	0.11	3
Lizinopril Lek	1	0.7	0.20	0.09	2
	2	0.8	0.26	0.09	4
Optimon	1	0.5 (0.52)	0.26	0.10	3
	2	0.5 (0.54)	0.45	0.31	3
	3	0.6 (0.61)	0.07	0.07	1
	4	0.6 (0.65)	0.08	0.08	1
	5	0.7	0.20	0.10	2
	6	0.8	0.22	0.12	2
Skopryl	1	0.1	0.41	0.05	6
	2	0.2	0.41	0.05	6
	3	0.6	0.35	0.08	4
	4	0.7	0.16	0.08	3
	5	0.8	0.38	0.12	4
	6	1.0 (0.98)	0.20	0.04	5
	7	1.0 (0.98)	0.27	0.11	4
Vitopril	1	0.2 (0.20)	0.37	0.07	8
	2	0.2 (0.20)	0.42	0.09	8
	3	0.2 (0.20)	0.36	0.06	7

IRV – index of the shelf life (explained in the section »Sampling«).

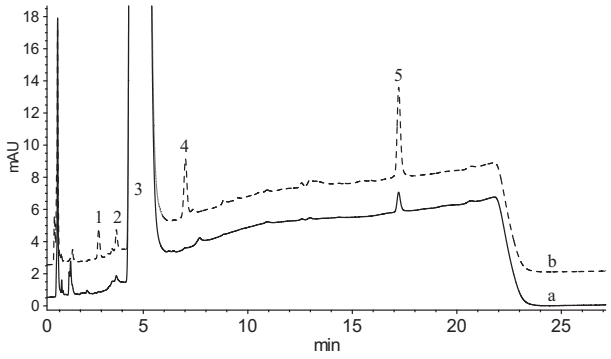

Fig. 2. Typical chromatogram presenting differences in impurity profiles of two drugs (»brands«): a) reference drug sample (Prinivil), b) sample number 3 referred to in Table 2 (Laaven). Peaks marked: 1 – unknown, 2 – impurity A, 3 – lisinopril, 4 – impurity E, 5 – impurity C.

Impurity profiles for each individual drug (»brand«) were found to differ mutually in their type and number of impurities present. Figure 2 presents specific chromatograms of two samples, showing the difference in impurity profiles and providing, at the same time, an example of a typical sample solution chromatogram.

Of the known specified impurities in the analyzed drugs, impurities A, C and E were present, with only the impurity C (S,S,S-diketopiperazine) appearing in all the analyzed drugs (»brand.«). Table 3 presents the results of the determined levels of total impurities and impurity C, as well as the number of impurities for each analyzed drug batch comprised with this study.

All the analyzed drugs meet the specification limits approved by the Agency for Medicinal Products and Medical Devices of Croatia (HALMED) in the procedure of granting marketing authorization in the Republic of Croatia (source: HALMED, confidential data).

Table 2 presents the obtained data on the average content of total impurities, impurities A, C, and E, as well as of total unknown impurities per individual drug (»brand«).

The comparison of impurity profiles and the levels of total impurities as well as of average values of the same parameters indicates to the existence of differences in the manufacturing processes and/or quality of the incoming raw materials among the drugs analyzed in this study. This is particularly well perceivable from the distribution graph of total impurities within the shelf life for each individual drug (»brand«) (Figure 3). The distribution graph of total impurities, which also includes the shelf life for each individual drug (IRV), shows not only variations in impurity levels from batch to batch of the same drug, but it also enables monitoring of the impact of the drug's age on possible increase of impurity levels. All the analysed drugs did not show clear trend of increasing of impurities within the shelf life. Observed differences in the distribution of impurities were somehow expected. On the other hand, the density of data indicate uniform quality of the medicinal products as regards purity. In other words, none of the analysed drugs stands

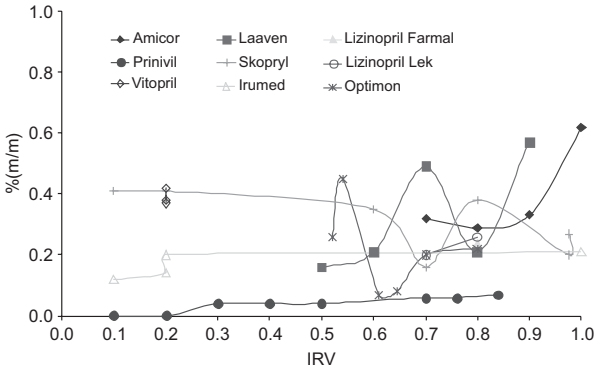

Fig. 3. Distribution of total impurities content within drug shelf life. IRV – index of the shelf life (explained in the section »Sampling«).

out from others with its impurity. Total impurities among analysed drugs amount up to 0.6% (m/m), which is within safe ranges of the specification limits that most manufacturers have set at 2.0% (m/m). The original drug stands out with its stability at a low impurity level from batch to batch and within shelf life.

Compared to its generic copies, the original drug has the lowest values and also the lowest variability of the monitored parameters. The characteristic impurity C appearing in all the analyzed preparations has the lowest levels in the original drug and shows the trend of a very mild increase. It is present in other preparations in higher amounts in relation to the original drug, without any clear trend of increasing which is probably surpassed by the variability among the batches. The impurity C is also quantitatively most represented among the detected impurities for most of the analysed drugs, with its levels showing similar distribution within the shelf life of the individual drug as well as the levels of total impurities. These results indicate a greater relevance of controlling the impurity C in the medicinal product in relation to other impurities, since changes in its levels have the greatest impact on the content of total impurities. This conclusion also complies with stability reports of the finished product in registration dossiers of medicinal products, as well as with specification limits within shelf life that were set at a significantly higher level for the impurity C (1.0–2.0 %) as the main degradation product.

The range of differences found in the purity of the tested drugs is not great, which is supported by the fact that level of the highest unknown impurity in any of the drugs does not exceed 0.10%.

Conclusion

One of the principal aims of the routine drug quality (purity) control is to verify whether the drug is meeting specification limits individually set forth for every pharmaceutical[11]. The general rule is that a generic drug should be of a comparable quality in relation to the original (reference) one; accordingly, specification limits stated for the generic drug should as the rule observe the levels specified for the reference drug[3,12]. The criteria specifying the allowed limits for impurities in an active substance and finished product are harmonized[10] and set forth within the so-called safe ranges not expected to affect the safety of drug administration. Although the manufacturers meet the mentioned criteria in the procedure of obtaining marketing authorization, there are still frequently significant differences among the medicinal products as regarding both the specification limits within the shelf life and the quality itself. The conclusion is also supported by the results of this study which, unlike routine control, was mainly aiming at the comparison of drugs of lisinopril tablets according to the parameter of purity as the key parameter essential for the safety of drug administration. According to the results of the study, the original drug stands out from all the generic preparations with its purity, but it is the general conclusion that all the analyzed drugs are, in the matter of impurities, within safe ranges of the specification limits. Therefore, it is not expected for the administration of the original drug, as compared to any of the generic drugs, to be safer for the patient.

This target comparison of purity within the shelf life indicates that there are certain differences in the quality of incoming raw materials and/or manufacturing processes among the medicinal drugs of lisinopril tablets. Although variability in the manufacturing process is anticipated, more significant variations in impurity levels from batch to batch may indicate to an inadequately controlled or designed manufacturing process of the active substance and/or medicinal product[3]. According to the results of this study, there were no substantial differences in impurity levels from batch to batch among analysed drugs. However, the lowest levels and the minimum variability of impurities in the original drug suggest that the overall manufacturing process (including the active substance, incoming raw materials, formulation and packaging of finished product) of the manufacturer of the original drug is to certain degree more stable compared to other manufacturers.

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ISPITIVANJE ČISTOĆE GENERIČKIH TABLETA LIZINOPRILA NA HRVATSKOM TRŽIŠTU

S A Ž E T A K

U kontekstu pojave sve većeg broja generičkih lijekova provedena je komparativna studija koja je uključila odobrene pripravke lizinoprila u obliku tableta koji se nalaze na hrvatskom tržištu u svrhu usporedbe čistoće generičkih pripravaka u odnosu na originalni lijek. Analizirano je više serija svakog pojedinog lijeka u različitoj fazi roka valjanosti. Određivanje onečišćenja provedeno je metodom tekućinske kromatografije visoke djelotvornosti (HPLC). Pokazano je da je profil onečišćenja specifičan za svaki pojedini lijek. Utvrđeno je da originalni lijek u usporedbi s generičkim paralelama ima najniže vrijednosti kao i najmanju varijabilnost svih promatranih parametara – vrsta, broj i maseni udio onečišćenja u odnosu na djelatnu tvar – što upućuje na zaključak da je proizvodni proces proizvođača originalnog lijeka u određenoj mjeri stabilniji u odnosu na ostale proizvođače. Karakteristično onečišćenje C, koje se pojavljuje u svim analiziranim pripravcima, najmanje razine ima u originalnom lijeku, dok udio najvećeg nepoznatog onečišćenja ne prelazi 0,10% u nijednom analiziranom pripravku. Iako se originalni lijek izdvaja većom čistoćom od svih generičkih pripravaka, opći je zaključak da su svi analizirani lijekovi po pitanju onečišćenja u sigurnim područjima specifikacijskih granica, te se stoga ne očekuje da će, u slučaju tableta lizinoprila, primjena originalnog lijeka u usporedbi s bilo kojim generičkim lijekom za pacijenta biti sigurnija.