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Original research paper

Dissolution profiles of perindopril and indapamide in their fixed-dose formulations by a new HPLC method and different mathematical approaches

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A new HPLC method was introduced and validated for simultaneous determination of perindopril and indapamide. Validation procedure included specificity, sensitivity, robustness, stability, linearity, precision and accuracy. The method was used for the dissolution test of perindopril and indapamide in three fixed-dose formulations. The dissolution procedure was optimized using different media, different pH of the buffer, surfactants, paddle speed and temperature. Similarity of dissolution profiles was estimated using different model-independent and model-dependent methods and, additionally, by principal component analysis (PCA). Also, some kinetic models were checked for dissolved amounts of drugs as a function of time.

Keywords: perindopril, indapamide, dissolution profiles, model-independent methods, model-dependent methods, PCA

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One example in the area of anti-hypertensive polytherapy is the use of an angiotensin converting enzyme inhibitor, *e.g.*, perindopril, and a diuretic, *e.g.*, indapamide (Fig. 1) in one fixed-dose formulation (1).

Bearing in mind previous reports concerning simultaneous determination of perindopril and indapamide, some spectrophotometric methods were developed (2–5). Also, a few HPLC methods were elaborated and validated in the range of official requirements (5–9). However, none of the above methods was applied to the dissolution study of perindopril and indapamide in their fixed-dose combinations.

A dissolution test is necessary to control the product properties within a batch and between batches. It is also needed in bioequivalence studies where similarity of dissolution profiles between a test product and a reference product should be demonstrated (10).

A wide range of methods are available for comparison of dissolution profile data (11–14). There are also official recommendations in this area (15, 16). However, there is no

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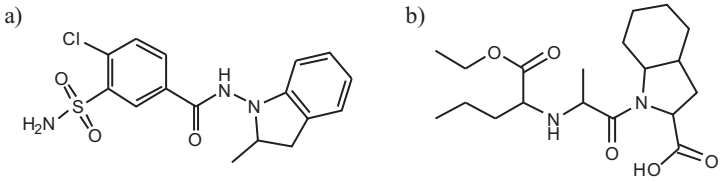

Fig. 1. Chemical structures of: a) indapamide and b) perindopril.

agreement about the best method. Some suggestions from the literature point to the necessity of stricter criteria on the difference allowed between two dissolution profiles (11, 17). Therefore, the question of the size of difference between the test and reference profiles should be allowed has a practical as well as scientific significance. Successful resolution of this issue may lead to the development of more appropriate methods for the comparison of dissolution profile data.

Thus, the first goal of the present study was to elaborate a new reliable HPLC method and a new optimized dissolution test for the simultaneous determination of perindopril and indapamide. The second goal was to compare the dissolution profiles of three fixed-dose formulations by several mathematical methods, trying to indicate the differences between them.

EXPERIMENTAL

Materials and reagents

Perindopril arginine (Oril Industrie, France) was obtained as a gift from Anpharm (Poland). Perindopril erbumine (*tert*-butyl amine) and indapamide were purchased from Sigma-Aldrich Chemicals (USA). Three fixed-dose formulations, *i.e.* Noliprel Forte® (NF) containing 5.0 mg of perindopril arginine and 1.25 mg of indapamide from Anpharm and Tertensif Kombi® (TK) (Servier, France), and Co-Prenessa® (CP) containing 4.0 mg of perindopril erbumine and 1.25 mg of indapamide from Krka (Poland) were used. The NF and TK tablets contained the same excipients, *i.e.* lactose monohydrate, magnesium stearate, maltodextrin, anhydrous colloidal silicon dioxide, sodium carboxymethyl starch type A, glycerol, hypromelose, Macrogol 6000 and titanium dioxide. Excipients for the CP tablets were microcrystalline cellulose, lactose monohydrate, sodium bicarbonate and colloidal silicon dioxide.

All solvents for chromatography, cetylpyridinium chloride (CPC) and Tween 80, were purchased from E. Merck (Germany). All other chemicals were supplied by Sigma-Aldrich Chemicals. Buffers of pH 2.6, 3.0 and 3.4 were prepared with 0.1 mol L^{-1} KH_2PO_4 and 85 % H_3PO_4. Buffers of pH 5.0, 5.5, 6.0 and 6.8 were prepared according to the *European Pharmacopoeia* (18).

Equipment

The HPLC system from Waters (USA) consisted of an Alliance e2695 separation module, model 515 isocratic pump and model 2998 PDA detector. It was controlled by the Em-

power Pro v.2.0 software. Separation was carried out on a LiChrospher® 100 RP18 column (125 mm×4.0 mm i.d., with a particle size of 5 μm) from E. Merck. For the dissolution study, an Evolution 6100 bathless dissolution system from Distek Inc. (USA) was used (paddle apparatus). The pH measurements were performed with a model HI9024C pH-meter from Hanna Instruments (Italy).

Dissolution studies

Dissolution was performed using 500 mL of phosphate buffer of pH 6.0 containing 0.5 % Tween 80 at 100 rpm and 37 °C. For every individual assay, two respective tablets were used to gain a concentration over the linearity range. At the time intervals 10, 20, 30, 40, 50 and 60 min, the samples were taken, filtered by nylon membrane filters (0.45 μm) and analyzed by the proposed HPLC method.

Stability in dissolution medium

Samples of perindopril and indapamide in the dissolution medium were heated in a water bath at 37 °C under continuous stirring. Respective volumes were taken at time intervals of 30, 60 and 90 min, diluted with the mobile phase to gain a concentration over the linearity range and analyzed by the proposed HPLC method for the presence of some additional peaks or changes of the existing ones.

Calculations and software

The obtained dissolutions profiles of perindopril and indapamide were compared by the model-independent methods, *i.e.* difference and similarity factors f_1 and f_2, Rescigno indices ξ_1 and ξ_2, Mahalanobis distance (MD), the area under the dissolution curve (*AUC*) and the mean residence time of drug molecules in the dosage form (*MRT*). In addition, principal component analysis (PCA) was applied to visualize the differences between three fixed-dose formulations.

Also, a number of kinetic models with dissolved amounts of drugs as a function of time were constructed and compared with the Akaike Information Criterion (*AIC*). The model-dependent methods, *i.e.*, the maximum fraction of the drug released at infinite time (F_{max}), Weibull method and the release rate constant (k_1) were used for further comparison. The Rescigno indices, *AUC* and *MRT* as well as *AIC* values were calculated based on the theoretical basis and equations proposed by Adams *et al.* (17) and Zhang *et al.* (19). All computations for the above parameters as well as PCA were performed using a free GNU R computational environment. The f_1 and f_2, MD as well as Weibull method were from Statistica® v. 10.0 containing Dissolution Profiles module (StatSoft, Poland).

HPLC

The mobile phase containing acetonitrile, methanol and phosphate buffer of pH 3.0 (50:3:47, *V*/*V*/*V*) was used. It was filtered through the nylon membrane filter (0.45 μm) and degassed prior to use. Separation was carried out on a LiChrospher® 100 RP18 column with a flow rate of the mobile phase equal 0.7 mL min^{-1} while the temperature of the column

was 30 °C. The working solutions (20 μL volumes) were injected and monitored spectrophotometrically at 210 nm.

Solutions

Perindopril and indapamide stock solutions were prepared by dissolving 10 mg of pure compounds in methanol to obtain a concentration of 1 mg mL^{-1} and then by diluting with methanol 10 times. All working solutions were prepared by respective diluting the above solutions with the mobile phase.

Validation

HPLC method was validated for specificity, robustness, stability, linearity, precision and accuracy, according to ICH guidelines (20).

Stability in working solutions. – The working solutions at the concentration of 40 μg mL^{-1} of perindopril and 10 μg mL^{-1} of indapamide were stored at temperature of 25 °C for 6, 12, 24 and 48 h in tightly capped volumetric flasks. The stability of drugs was then checked by analyzing chromatograms for the presence of some additional peaks or changes of the existing ones.

Robustness. – Robustness of HPLC method was checked after deliberate alterations of the buffer in the mobile phase (in the range 2.9–3.1), the flow rate of the mobile phase (in the range 0.5–0.9 mL min^{-1}) and the column temperature (in the range 21–23 °C), using one concentration of perindopril and indapamide over the linearity range (40 μg mL^{-1} of perindopril and 10 μg mL^{-1} of indapamide). Robustness of the dissolution was checked after small alterations of % Tween 80 in the medium (in the range 0.4–0.6 %), pH of the buffer in the medium (in the range 5.9–6.1) and temperature (in the range 36–38 °C), using tablets from one two-component formulation (TK). The effects of a single factor at three levels, nominal, lower and upper, were estimated in individual sets. Resolution factor (R_s) between perindopril and indapamide as well as recoveries of the drugs were then determined for robustness testing.

Calibration and limiting values. – Calibration solutions were prepared over the concentration ranges 12–60 μg mL^{-1} for perindopril and 3–15 μg mL^{-1} for indapamide. Injection of 20 μL of each working solution was repeated six times for each sample. The peak areas were then plotted against the corresponding drug concentrations. Limit of detection (*LOD*) and limit of quantification (*LOQ*) were calculated from the equations of $LOD = 3.3 \times SD/a$ and $LOQ = 10 \times SD/a$, using the SD of the intercept of the regression line in proximity of *LOD* and the slope of the calibration curve (*a*) (20).

Precision. – Precision of the method was evaluated by injecting the working solutions at three different concentrations (24, 40, 56 μg $mL^{-1}$of perindopril and 6, 10, 14 μg mL^{-1} of indapamide). These solutions were analyzed three times within the same day (within-day precision) and three times over a period of three days (day-to-day precision).

Accuracy. – Accuracy was evaluated by the standard addition method at three levels. Weighed portions of powdered tablets containing 8 or 10 mg of perindopril and 2.5 mg of indapamide were transferred to 100-mL flasks, sonicated for 15 min, diluted to the mark

and filtered through nylon membrane filters (0.45 μm). Then, 1.2-, 2.0- and 2.8-mL volumes of these extracts were fortified with 2.0-mL volumes of the standard solutions of perindopril and indapamide (0.1 mg mL^{-1}), diluted to 10 mL and analyzed by HPLC method in the same day and three times over a period of three days. The assay was repeated three times at each level of addition. The results were estimated by calculating the respective recoveries of drugs.

Assay in tablets

Weighed portions of powdered tablets containing 8 or 10 mg of perindopril and 2.5 mg of indapamide were transferred to 100-mL flasks, sonicated for 15 min, diluted to the mark and filtered through nylon membrane filters (0.45 μm). Then, 3.0-mL volumes were diluted to 10 mL and analyzed by HPLC. The assay was repeated six times, individually weighing the respective tablet powders. The results were estimated by checking if the determined concentrations of the compounds were inside respective 95 % confidence intervals as well as by calculating RSD values.

RESULTS AND DISCUSSION

Chromatography optimization

Mobile phases containing acetonitrile, methanol and different phosphate buffers (pH 2.6, 3.0 and 3.4) were examined. Also, the effects of the flow rate of the mobile phase (0.5–1.0 mL min^{-1}) and column temperature (25–40 °C) were checked.

The mobile phase containing acetonitrile, methanol and phosphate buffer of pH 3.0 (50:3:47, *V*/*V*/*V*) with the flow rate of 0.7 mL min^{-1} was finally used at 30 °C. As a result, well defined and resolved peaks with mean retention times of ca. 2.9 and 4.6 min, for perindopril and indapamide, respectively, were obtained (Fig. 2).

The chromatographic system was checked by repetitively injecting the drug solution at concentration level of 40 μg mL^{-1} for perindopril and 10 μg mL^{-1} for indapamide and then by estimating parameters such as peak symmetry, resolution factor and theoretical plate number. All results were satisfactory and indicated sufficient effectiveness of the system for perindopril and indapamide assessment (Table I).

Validation

Specificity. – Specificity of the method was proven by the lack of interference peaks from excipients present in formulations as well as by the peak-purity function. Chromatograms obtained from two-component tablets were almost identical to those obtained from the standard solutions of perindopril and indapamide (Fig. 2).

Robustness. – Robustness of the method was checked after deliberate alterations of some operational parameters including pH of the buffer in the mobile phase, the flow rate of the mobile phase and column temperature. It was shown that RSD from recoveries was 0.7 % for perindopril and in the 0.4–0.5 % for indapamide. At the same time, the RSD for the R_s ranged from 1.3 to 1.5 %. All results are presented in Table II.

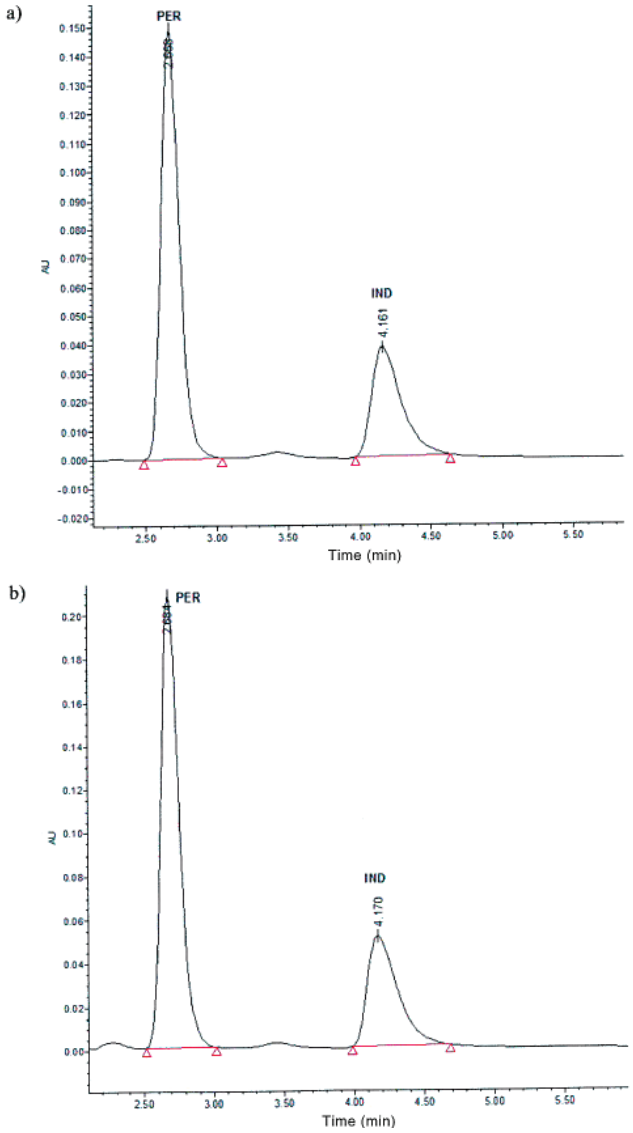

Fig. 2. Representative chromatograms of indapamide (IND) and perindopril (PER) from: a) standard solution and b) from the marketed formulation. HPLC conditions are described in the text.

Stability. – The drugs resolved in methanol were stable when stored at 25 °C for 48 h and no additional peaks or changes of the existing ones were observed in the chromatograms. Further, the samples in the dissolution medium, heated at 37 °C for 90 min did not

Table I. Chromatographic parameters of the proposed method

Parameter	Perindopril (n = 6)	Indapamide (n = 6)
Retention time (min)	2.9	4.6
Peak width at $h_{½}$ (min)	0.1	0.3
Peak width at the base (min)	0.3	0.6
Asymmetry factor	1.19	1.23
Resolution factor	–	3.58
Theoretical plate number (N m^{-1})	37273	10418

show any significant change. Recoveries of both drugs from stored solutions, in comparison with the respective standards, were ranged from 98.5 to 101.5 %.

Table II. Robustness of the HPLC method and the dissolution procedure

Operational parameter		Recovery of perindopril (%)[a]	RSD (%)	Recovery of indapamide (%)[a]	RSD (%)[b]	R_s[a]	RSD (%)[b]
pH of the buffer in the mobile phase	2.9	98.01 ± 0.71	0.72	101.0 ± 0.39	0.37	3.59 ± 0.04	1.25
	3.0	98.45 ± 0.78		100.6 ± 0.45		3.58 ± 0.04	
	3.1	99.49 ± 0.67		100.8 ± 0.34		3.63 ± 0.05	
Flow rate of the mobile phase (mL min^{-1})	0.5	99.35 ± 0.75	0.73	100.1 ± 0.41	0.46	3.67 ± 0.06	1.47
	0.7	98.45 ± 0.65		100.6 ± 0.43		3.58 ± 0.05	
	0.9	100.0 ± 0.67		101.4 ± 0.58		3.54 ± 0.05	
Column temperature (°C)	21	99.67 ± 0.73	0.66	100.8 ± 0.53	0.54	3.68 ± 0.06	1.36
	22	98.45 ± 0.67		100.6 ± 0.59		3.58 ± 0.03	
	23	99.89 ± 0.58		99.78 ± 0.53		3.61 ± 0.05	
pH of the buffer in the dissolution medium	5.9	84.67 ± 0.45	0.61	85.23 ± 0.52	0.58	–	
	6.0	84.25 ± 0.53		85.05 ± 0.48		–	
	6.1	85.10 ± 0.58		85.34 ± 0.52		–	
% Tween 80 in the dissolution medium	0.4	85.34 ± 0.43	0.56	84.98 ± 0.48	0.58	–	
	0.5	84.25 ± 0.56		85.05 ± 0.49		–	
	0.6	84.89 ± 0.47		85.23 ± 0.53		–	
Dissolution medium temperature (°C)	36	85.31 ± 0.47	0.61	85.46 ± 0.53	0.55	–	
	37	84.25 ± 0.54		85.05 ± 0.42		–	
	38	84.56 ± 0.56		84.95 ± 0.48		–	

[a] Mean ± *SD*, n = 3
[b] n = 9

Table III. Linearity study of the HPLC method for perindopril erbumine, perindopril arginine and indapamide for each concentration

Linearity range (μg mL^{-1})	Equation	Slope RSD (%)	Intercept RSD (%)	R^2	*F* (Snedecor)	*p*	*LOD* (μg mL^{-1})	*LOQ* (μg mL^{-1})
Perindopril erbumine 12–60	y = 65782x + 42240	0.3	16.9	0.9988	34212	< 0.001	3.57	10.83
Perindopril arginine 12–60	y = 67104x – 8185	0.3	15.9	0.9995	87116	< 0.001	3.23	11.93
Indapamide 3–15	y = 100160x + 44942	0.6	25.5	0.9985	26594	< 0.001	0.38	1.15

[a] $n = 6$

Linearity. – The results of the linearity study with their statistical analysis are given in Table III. For perindopril erbumine and perindopril arginine, the method was linear over the range from 12 to 60 μg mL^{-1} with the coefficients of determination (R^2) of 0.9988 and 0.9995, respectively. The achieved *LOD* and *LOQ* values were 3.57 and 10.83 μg mL^{-1} for perindopril erbumine, and 3.23 and 11.93 μg mL^{-1} for perindopril arginine. For indapamide, the method was linear over the range from 3 to 15 μg mL^{-1} with the R^2 of 0.9985. The achieved values of *LOD* and *LOQ* were 0.38 and 1.15 μg mL^{-1}. Linearity was also assessed by defining the residuals of regression. The obtained residual plots confirmed that regression residuals did not present a visible trend and were randomly scattered. The Shapiro Wilk test for normality did not reject the hypothesis that residuals were normally distributed.

Precision. – The results of the precision study are given in Table IV. The repeatability (within-day precision) expressed as RSD was 0.1 % for perindopril erbumine. RSDs for indapamide were in the range from 0.1 to 0.6 %. The intermediate (day-to-day) precision was up to 0.4 % of perindopril erbumine, while for perindopril arginine it was 0.3 %. The respective values for indapamide were 0.5 %.

Accuracy. – Accuracy of the method was assessed by determining of perindopril and indapamide in fortified samples at three levels of addition (Table V). For perindopril, recovery ranged from 96.9 to 99.0 % for the lowest and highest drug concentration, with the mean RSD 0.7–2.2 %. For indapamide, recovery ranged from 101.1 to 100.9 % for the lowest and highest drug concentration, with the mean day-to-day RSD of 1.1 %.

Assay in tablets

Precision of the method was also checked by the determining of perindopril and indapamide in the tablets with respective RSD values of 0.2–0.4 and 0.4–0.8 %.

Dissolution study

The choice of optimal pH of dissolution medium was difficult due to significant differences in chemical properties of perindopril and indapamide. Therefore, different phosphate buffers (pH 5.0, 5.5, 6.0 and 6.8) were examined as dissolution media. The effect of

Table IV. Precision of the HPLC method for perindopril erbumine, perindopril arginine and indapamide

Expected concentration ($\mu g\ mL^{-1}$)	Within-a-day		Day-to-day	
	Determined[a]	RSD (%)	Determined[b]	RSD (%)
Perindopril erbumine				
24.0	23.25 ± 0.02	0.07	23.33 ± 0.09	0.37
40.0	38.65 ± 0.02	0.05	38.69 ± 0.05	0.13
56.0	54.40 ± 0.04	0.07	54.39 ± 0.01	0.02
Perindopril arginine				
24.0	23.70 ± 0.08	0.33	23.66 ± 0.08	0.34
40.0	39.08 ± 0.17	0.45	39.08 ± 0.11	0.29
56.0	54.56 ± 0.06	0.12	54.48 ± 0.15	0.28
Indapamide				
6.00	5.94 ± 0.01	0.10	5.93 ± 0.03	0.48
10.0	10.07 ± 0.02	0.21	10.09 ± 0.02	0.19
14.0	14.03 ± 0.09	0.63	14.06 ± 0.07	0.50

Mean ± SD ($\mu g\ mL^{-1}$): [a] $n = 3$, [b] $n = 9$.

the rotation speed of the paddle was also examined in the range of 50–100 rpm according to the *European Pharmacopoeia* (18).

The best results for both perindopril and indapamide were obtained using the buffer of pH 6.0 at 100 rpm though some individual results were below 80 %. According to *USP* (21), the use of surfactants is allowed to obtain higher dissolution values. Therefore, two different surfactants, cationic CPC and non-ionic Tween 80 in concentrations 0.02–0.5 %, were tried. Finally, phosphate buffer of pH 6.0 containing 0.5 % Tween 80 was used for all dissolution tests.

Robustness. – Robustness of the dissolution procedure was checked after deliberate alterations of % Tween 80 in the medium, pH of the buffer in the medium and temperature. Respective dissolution tests along with quantitative assays were performed in triplicate and areas of the drugs were recorded for further estimation. It was shown that these small changes of the parameters did not lead to significant changes of RSD recovery values. The RSD values were 0.6 % for perindopril and for indapamide (Table II), confirming the robustness of the described dissolution procedure.

Comparison of dissolution profiles

Percent dissolution of perindopril and indapamide as a function of time are presented in Fig. 3. In pairwise procedures discussed below, three fixed-dose formulations were

Table V. Accuracy of the HPLC method for perindopril and indapamide in fortified samples

Drug	Expected concentration ($\mu g\ mL^{-1}$)	*Within-a-day*		*Day-to-day*	
		Recovery[a]	RSD (%)	Recovery[b]	RSD (%)
Noliprel Forte®					
Perindopril	32.0	97.47 ± 0.77	0.81		
	40.0	98.52 ± 0.52	0.53	98.34 ± 0.79	0.80
	48.0	99.02 ± 0.51	0.51		
Indapamide	8.00	101.75 ± 1.10	1.07		
	10.0	100.76 ± 0.61	0.61	101.1 ± 1.16	1.14
	12.0	100.73 ± 0.57	0.57		
Tertensif Kombi®					
Perindopril	32.0	97.60 ± 2.33	2.40		
	40.0	97.29 ± 1.81	1.86	97.82 ± 0.67	0.69
	48.0	98.58 ± 0.85	0.86		
Indapamide	8.00	101.11 ± 1.10	1.07		
	10.0	101.02 ± 1.10	1.08	101.0 ± 1.07	1.05
	12.0	100.91 ± 1.18	1.17		
Co-Prenessa®					
Perindopril	28.0	96.89 ± 0.04	0.62		
	36.0	97.64 ± 0.44	0.45	98.51 ± 0.22	2.22
	44.0	98.79 ± 0.42	0.43		
Indapamide	8.00	101.50 ± 0.96	0.46		
	10.0	100.50 ± 0.46	0.33	100.87 ± 1.11	1.10
	12.0	100.67 ± 0.45	0.45		

Mean ± SD (%): [a] $n = 3$, [b] $n = 9$.

compared in pairs: NF versus TK, NF versus CP and TK versus CP. In each pair, the first formulation was considered as a test while the second as a reference product.

First, similarity between dissolution profiles was assessed by the model independent methods such as the difference factor f_1, the similarity factor f_2, Rescigno indices ξ_1 and ξ_2 and the MD method (Table VI).

The f_1 factor measures the percent error between two curves over all time points. The f_2 factor is a logarithmic transformation of the sum-squared error of differences between the

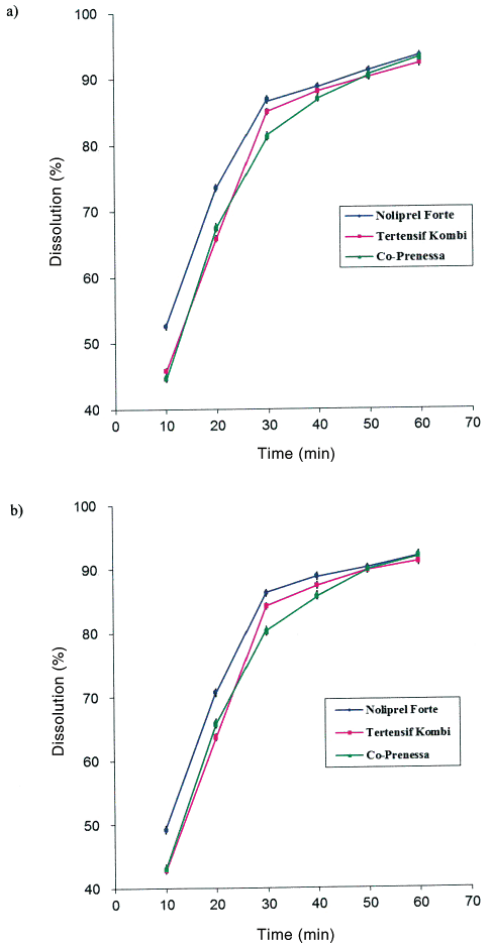

Fig. 3. Dissolution profiles of: a) indapamide and b) perindopril from Noliprel Forte®, Tertensif Kombi® and Co-Prenessa® tablets (mean ± SD, n = 12 at each time interval).

test and the reference products over all time points. It is known from the literature that these factors are sensitive to the measurements beyond 85 % dissolution (11, 13, 14). In the present work, the values of f_1 and f_2 were calculated twice, once for the dissolution results up to 40 min (the time at which the dissolution profiles nearly reached the final plateau) and up to 60 min (the time at which the dissolution process was completed). In the present work, all f_1 values are smaller than 15 and all f_2 values are higher than 50, indicating that the examined products show similar dissolution profiles of perindopril and indapamide. Because of its simplicity, f_2 method is recommended by EMA and FDA guidelines (15, 16). Nevertheless, it is not the optimal method mainly because of not taking into account the shape of the curve in a dissolution profile. It is also sensitive to the number of time points used (11, 12, 21).

The Rescigno indices can be thought of as functions of the weighted average of the vertical distances between the test and reference mean profiles at each time point. In the present work, all Rescigno indices obtained for perindopril and indapamide are close to zero, indicating that the examined pairs of formulations show similar dissolution profiles. The Rescigno indices do not exert any major advantages over f_1 or f_2 factors, with the exception that interchanging the products in pair does not alter their values. As with f_1 and f_2 factors, the Rescigno indices do not take into account the variability or correlation between respective dissolution time points (11, 15).

When the within-product variability has a coefficient of variation greater than 15 %, a multivariate confidence region procedure based on Mahalanobis distance (MD) is recommended (11, 12, 16). In our study, all values of the upper limit of the confidence interval (UPCI) are lower than the respective similarity limit (SL) indicating that all pairs of formulations have similar dissolution profiles. This approach is not as simple to interpret as the f_2 method. Also, the nature of the difference between the mean dissolution profiles is not strictly defined. This means that profiles with large differences at early time points and small differences at later time points may yield the same value for the MD as mean dissolution profiles with small differences at early time points and large differences at later time points (11, 12).

On the other hand, some model independent methods take into account the variability of dissolution curves, *e.g.* the methods based on *AUC* or *MRT* (12, 19). It was interesting to observe that these methods did not show similarity between three fixed-dose formulations, in contrast to other model-independent methods discussed above.

Further, PCA was used to compare the dissolution profiles of perindopril and indapamide. This method is very useful, especially to visualize data variability (14, 21). In Fig. 4, plots of the weighed scores of the first two PCs are presented for perindopril and indap-

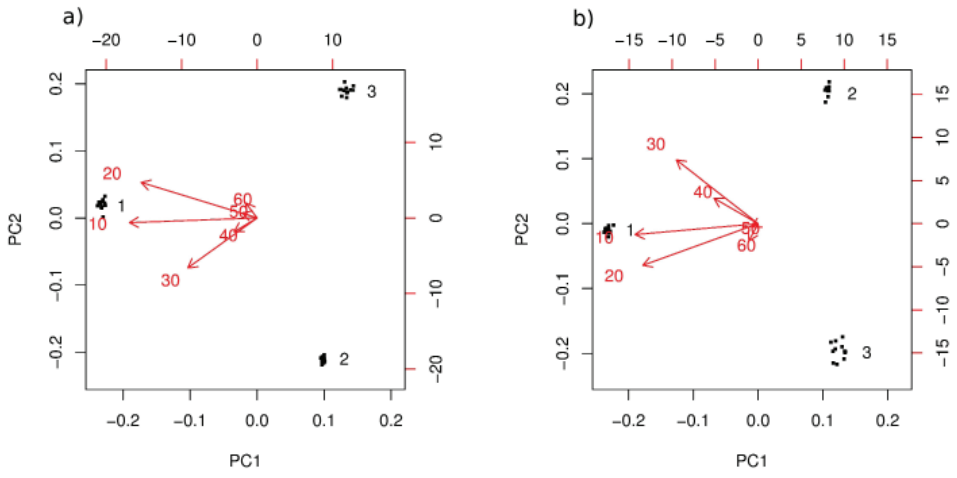

Fig. 4. PC1 *vs.* PC2 scores plots for: a) indapamide and b) perindopril dissolved from Noliprel Forte® (1), Tertensif Kombi® (2) and Co-Prenessa® (3) tablets.

Table VI. Comparison of the dissolution profiles of perindopril (P) and indapamide (I) from Noliprel Forte®, Tertensif Kombi® and Co-Prenessa® by the model-independent methods

Drug formulation		Model-independent method											
		f_1		f_2		Rescigno		Mahalanobis		*MRT*		*AUC*	
		40 min	60 min	40 min	60 min	ξ_1	ξ_2	ULCI	SL	*t*	*p*	*t*	*p*
P	NF × TK	5.7	3.8	65.1	69.1	0.018	0.024	125.1	663.4	33.217	$1.749\ 10^{-14}$[a]	288.14	$< 2.2\ 10^{-16}$[a]
	NF × CP	6.7	4.2	64.1	68.3	0.021	0.026	75.24	221.9	3.4151	0.002841[a]	107.49	$< 2.2\ 10^{-16}$[a]
	TK × CP	2.9	2.0	79.4	82.7	0.011	0.013	53.95	256.0	–19.271	$1.545\ 10^{-10}$[a]	16.712	$2.38\ 10^{-9}$[a]
I	NF × TK	5.7	3.9	63.7	67.7	0.019	0.025	135.9	382.9	44.301	$< 2.2\ 10^{-16}$[a]	191.10	$< 2.2\ 10^{-16}$[a]
	NF × CP	7.6	4.8	61.7	65.9	0.022	0.027	80.76	194.8	15.025	$2.335\ 10^{-10}$[a]	132.04	$< 2.2\ 10^{-16}$[a]
	TK × CP	2.7	1.9	81.1	84.2	0.010	0.012	59.08	221.3	–6.1717	$3.509\ 10^{-5}$[a]	22.561	$2.82\ 10^{-12}$[a]

[a] Dissolution profiles are not similar;
ULCI – the upper limit of the confidence interval; SI – the similarity limit; *AUC* – the area under dissolution curve, *MRT* – the mean residence time of the drug substance molecules in the dosage form.
NF – Noliprel Forte
TK – Tertensif Kombi
CP – Co-Prenessa

Table VII. The AIC values for the mathematical models (ref. 12) fitted to the dissolution profiles of perindopril

Model	Noliprel Forte®	Tertensif Kombi®	Co-Prenessa®
First-order	36.44415	34.77248	31.00554
First-order with F_{max}[a]	27.06262	33.34143	15.29032
First-order with T_{lag}[a]	35.29825	36.17412	28.62448
First-order with T_{lag}[a] and F_{max}[a]	28.91235	34.74951	17.27064
Gompertz	30.22312	35.85379	28.50177
Gompertz with F_{max}[a]	32.11875	37.12908	23.97992
Hixson-Crowell	43.60959	42.07886	42.00419
Hixson-Crowell with T_{lag}[a]	39.90886	40.28923	36.54190
Higuchi	47.47772	44.82222	42.94442
Higuchi with F_0[a]	43.63776	44.55590	41.46322
Higuchi with T_{lag}[a]	44.92026	45.32972	42.55526
Korsmeyer-Peppas	42.12413	43.61894	40.12649
Logistic 2	31.21082	36.22876	21.51761
Peppas-Sahlin	31.41325	35.52770	24.96117
Quadratic	45.37512	40.53122	42.01119
Quadratic with T_{lag}[a]	35.13124	35.11358	31.51846
Weibull 1	32.69823	35.68629	25.64291
Weibull 2	32.69823	36.85985	22.65522
Weibull 3	28.66803	34.14105	17.21210
Zero-order	59.12620	57.53103	57.19584
Zero-order with F_0[a]	46.79082	47.86347	45.73334
Zero-order with T_{lag}[a]	46.79082	47.86347	45.73334

[a]F_0 – initial fraction of the solution resulting from a burst release
F_{max} – maximum fraction of the drug released at infinite time
T_{lag} – the lag time prior to drug release

amide. The examined formulations are described as 1 (NF), 2 (TK) and 3 (CP) and each product is represented by 12 points. In the data set obtained for perindopril, the PC1 is explanatory to 84.3 % while PC2 described 15.5 % of the total variance. For indapamide, the respective values of PC1 and PC2 are 89.2 and 10.6 %. The plots for perindopril and indapamide show that along PC1, respective dissolution points from 1 (NF) are located far away from two other formulations 2 (TK) and 3 (CP). It seems that the dissolution profiles of both drugs for these three products are nor similar. Additionally, for both perindopril and indapamide, the points from formulation 1 have evidently different scores along PC2 than the points from formulations 2 and 3. It could be concluded that the dissolution profile for 1 has a different shape than profiles for 2 and 3, for both drugs.

Table VIII. The AIC values for the mathematical models (ref. 12) fitted to the dissolution profiles of indapamide

Model	Noliprel Forte®	Tertensif Kombi®	Co-Prenessa®
First-order	36.73912	34.59528	30.62983
First-order with F_{max}[a]	24.20855	31.64352	13.92008
First-order with T_{lag}[a]	33.30772	34.83239	27.36750
First-order with T_{lag}[a] and F_{max}[a]	25.73668	33.61148	15.49636
Gompertz	26.81570	35.13727	28.44597
Gompertz with F_{max}[a]	28.38519	36.04252	22.43291
Hixson-Crowell	43.86255	42.37242	41.85304
Hixson-Crowell with T_{lag}[a]	38.42262	39.16109	35.94854
Higuchi	48.34935	45.35483	43.65520
Higuchi with F_0[a]	42.28709	43.65211	41.36269
Higuchi with T_{lag}[a]	43.83206	44.65431	42.58625
Korsmeyer-Peppas	40.43366	42.46797	39.87341
Logistic 2	27.58080	35.24502	19.80382
Peppas-Sahlin	29.87055	34.94374	25.14767
Quadratic	47.55915	42.87547	43.15908
Quadratic with T_{lag}[a]	35.26422	34.85393	32.09568
Weibull 1	30.43932	34.10911	23.92524
Weibull 2	28.85522	35.54786	20.55119
Weibull 3	25.87895	33.43350	15.59772
Zero-order	59.67752	58.06473	57.60860
Zero-order with F_0[a]	45.58446	47.04676	45.59996
Zero-order with T_{lag}[a]	45.58446	47.04676	45.59996

[a]F_0 – initial fraction of the drug in the solution resulting from a burst release
F_{max}– maximum fraction of the drug released at infinite time
T_{lag} – lag time prior to drug release

In the next step, the model-dependent approaches were applied for comparisons. First, a number of kinetic models were constructed and then estimated with the *AIC* values (Tables VII, VIII). The *AIC* is a measure of the goodness of fit based on the maximum likelihood. When comparing several models for a given set of data, the model with the smallest *AIC* is regarded as giving the best fit (12). Considering the *AIC* for perindopril and indapamide, the preferred model was the first-order with F_{max}. The second best were the first-order with T_{lag}, F_{max} and Weibull 3-model for both perindopril and indapamide. In the next step, the F_{max}, Weibull and k_1 models were used for additive pairwise comparisons. It is known from the literature that linearization of dissolution profiles using the above models could better characterize the differences between these profiles. Especially, Weibull

Table IX. Comparison of the dissolution profiles of perindopril (P) and indapamide (I) from Noliprel Forte®, Tertensif Kombi® and Co-Prenessa® by the model-dependent methods

Drug/Formulations		Model-dependent method				
		k_1		F_{max}		Weibull model
		t	*p*	*t*	*p*	*p*
P	NF × TK	164.996	< 2.2 10^{-16}[a]	–68.467	< 2.2 10^{-16}[a]	< 0.05[a]
	NF × CP	124.216	< 2.2 10^{-16}[a]	–25.416	1.35 10^{-14}[a]	< 0.05[a]
	TK × CP	–0.8974	0.3844	12.028	1.761 10^{-8}[a]	< 0.05[a]
I	NF × TK	306.98	< 2.2 10^{-16}[a]	–89.200	< 2.2 10^{-16}[a]	< 0.05[a]
	NF × CP	178.45	< 2.2 10^{-16}[a]	–37285	4.946 10^{-15}[a]	< 0.05[a]
	TK × CP	20.693	2.24 10^{-10}[a]	–3.2847	0.006506[a]	< 0.05[a]

[a] dissolution profiles are not similar
k_1 – the first order release constant
F_{max} – maximum fraction of the drug released at infinite time

parameters are frequently used to compare the dissolution profiles between the reference and test products (17, 19). When all these methods were used for NF, TK and CP formulations, the dissolution profiles of both perindopril and indapamide were shown to be dissimilar (Table IX).

Overall, only some model-independent methods, *i.e.*, f_1 and f_2, Rescigno and MD showed similarity of perindopril and indapamide profiles, when dissolved from three fixed-dose formulations. Other model-independent procedures taking into account the variability of the curves (*AUC* and *MRT* methods) and all model-dependent methods as well as PCA did not show similarity for the same experimental data. Therefore, it was concluded that some recommended methods, especially the model-independent ones may be insufficient. These methods allow the use of one arbitrarily chosen value for the difference allowed between the test and reference products (17). Our present results as well as some literature data (11, 14) suggest that differences both in the level and the shape of dissolution curves are important when comparing profiles.

CONCLUSIONS

Quantitative data including dissolution profiles were obtained for three fixed-dose formulations containing perindopril and indapamide using a new validated HPLC method. Since there is no suitable method in the literature, the procedure presented here can be used as a reliable quality control test for such combined formulations. In addition, different mathematical approaches were used to compare dissolution profiles and many differences were found. It was concluded that discrimination between profiles was found when

data variability within each formulation as well as the shape and size of the dissolution curves were taken into account. Because the three examined fixed-dose formulations were obtained on the market, the most important question is what kind of difference between the dissolution was considered to be of practical importance, *e.g.* having an impact on *in vivo* performance of a respective product.

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