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Chemical Industry & Chemical Engineering Quarterly 18 (1) 19–25(2012) CI&CEQ

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SCIENTIFIC PAPER

UDC 543.544.5:661.12

DOI 10.2298/CICEQ110628042J

VALIDATION OF A DISSOLUTION METHOD WITH RP-HPLC ANALYSIS FOR PERINDOPRIL ERBUMINE AND INDAPAMIDE COMBINATION TABLET

A dissolution method with high performance liquid chromatography (HPLC) analysis was validated for perindopril erbumine and indapamide in combination tablet formulation. The method was validated to meet requirements for a global regulatory filing and this validation included specificity, linearity, accuracy, precision, range, robustness and solution stability studies. The dissolution method, which uses a USP apparatus 1 with basket rotating at 100 rpm, 1000 ml of phosphate buffer, pH 6.8, as the dissolution medium, and reversed-phased HPLC was carried out at 50 °C on a 4.6 mm×250 mm×5 μm cyano column that contained USP packing L1 with acetonitrile:buffer 40:60 (v/v), pH 2.8, as mobile phase. UV detector was set at 225 nm. The method was found to be selective, linear, accurate and precise in the specified ranges. Intra-day and inter-day variability for method was <2% RSD. This method was successfully used for quantification of perindopril erbumine and indapamide combination tablet formulations.

Key words: perindopril erbumine (PE); indapamide; RP-HPLC; dissolution; validation.

Dissolution test has emerged in the pharmaceutical field as a very important tool to characterize drug product performance [1]. It provides measurements of the bioavailability of a drug as well as can demonstrate bioequivalence from batch-to-batch. Besides, dissolution is a requirement for regulatory approval for product marketing and is a vital component of the overall quality control program [2,3].

The dissolution testing is used to guide development of new drug products and to assess lot-to-lot variability of drug products. Dissolution methods, as well as other analytical methods, are validated to ensure they are suitable for their intended use and give accurate and reliable data. Guidance on validation characteristics and considerations has been published [3,4]. Validation of a dissolution method typically involves validation of the end analysis method for specificity, linearity, accuracy, precision, range, robustness and solution stability studies. A number of papers have been published detailing validation studies for the dissolution analysis method [5-10].

Indapamide is chemically benzamide, 3-(aminosulfonyl)-4-chloro-*N*-(2,3-dihydro-2-methyl-1*H*-4-indol--1-yl)chloro-*N*-[(2*RS*)-2-methyl-2,3-dihydro-1*H*-indol--1-yl]-3-sulphamoylbenzamide (Figure 1) [11]. Indapamide (molecular formula: $C_{16}H_{16}ClN_3O_3S$, molecular weight: 365.84 g mol^{-1}) belongs to antihypertensive category listed in BP and USP. Indapamide reduces blood pressure by a vasodilator or a diuretic action [12].

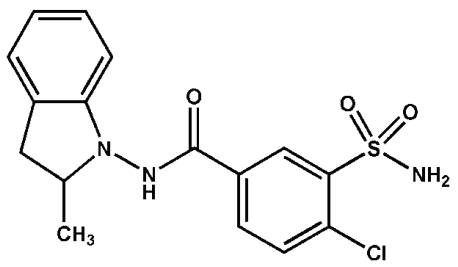

Figure 1. The chemical structure of indapamide.

Perindopril erbumine is chemically 2-methylpropan-2-amine (2*S*,3*aS*,7*aS*)-1-{(2*S*)-2-[9((1*S*)-1-(ethoxycarbonyl)butyl)amino]propanoyl}-octahydro-1*H*-indole-

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Paper received: 28 June, 2011
Paper revised: 13 September, 2011
Paper accepted: 13 September, 2011

-2-carboxylate (Figure 2). Perindopril erbumine (molecular formula: $C_{19}H_{32}N_2O_5$, molecular weight: 441.6 g mol^{-1}) belongs to antihypertensive category listed in BP. Perindopril is an ACE inhibitor. It works by blocking the action of angiotensin converting enzyme (ACE) [13]. The literature survey revealed two HPLC methods were reported on both the drugs in combination [14-15].

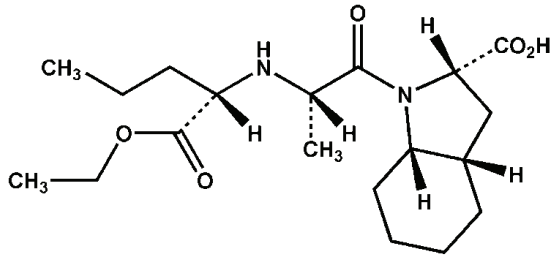

Figure 2. The chemical structure of perindopril erbumine.

This paper describes the validation of a dissolution method with RP-HPLC analysis for PE and indapamide tablets as per current ICH [16,17] and FDA [18] guidelines. A simple and specific RP-HPLC method was developed for the quality control analysis of PE and indapamide in dissolution samples.

EXPERIMENTAL

Materials

Potassium dihydrogen phosphate, NaOH, both reagent grade, and acetonitrile (HPLC grade) were obtained from Merck Chemicals. Orthophosphoric acid (AR Grade) was obtained from (LOBA Chemicals). Purified water for dissolution and chromatography were obtained from a Milli-Q purification unit (Millipore, Milford, MA, USA). Perindopril ebrumine and indapamide drug was obtained as a gift sample (Mepro Pharmaceuticals Pvt. Ltd., Surendranagar, India).

Instrumentation

For all dissolution experiments, an Electrolab TDT-08L (Mumbai, India) was used. For preparation of standards and mobile phase an analytical balance (Shimadzu Aux 120; Japan) and pH meter (Systronics pH system 362; India) were used. For all post-dissolution analyses a Shimadzu HPLC system LC-2010CHT version 3.10 (Shimadzu; Japan) was used.

Methods

In vitro dissolution test conditions

Dissolution testing was performed in compliance with USP {711} using apparatus 1 with basket [19]. A dissolution medium of phosphate buffer, pH 6.8, a basket speed of 100 rpm was selected to indapamide tablet as per USP. The media volume used was 1000 mL. The medium, which was degassed under house vacuum, was maintained at 37±0.5 °C. Manual sampling was performed using 10 mL aliquots. These solutions were immediately filtered using a Whatman 1# filter paper. The first few of sample were discarded prior to collecting the sample for analysis.

HPLC Method

An HPLC method with UV detection was selected for the method of analysis. The reversed-phase procedure utilized a Phenomenex Luna C_{18} column (10 μm; 250 mm×4.6 mm i.d.) and UV detection at 225 nm. This wavelength was selected because it is a UV maximum and provides the sensitivity needed for quantitation of the low drug concentration in the dissolution samples. The column temperature was maintained at 50 °C. The mobile phase contained acetonitrile, buffer, pH 2.8 (40:60, v/v, respectively). The flow rate was 0.5 mL/min for 19 min with an injection volume of 50 μL. A standard solution of active pharmaceutical ingredient (API) was prepared in diluting solution (acetonitrile:water, 40:60 v/v, respectively). This standard solution contained 100% of the final assay concentration of drug PE (≈ 4 μg/mL) and concentration of drug indapamide (≈ 1.25 μg/mL).

Sample preparation

Weigh and crush 20 tablets. Take crushed powder equivalent to 1.6 mg of perindopril erbumine and 0.5 mg of indapamide and transfer into a 50 mL volumetric flask. Add 35 mL diluting solution and sonicate for 20 min to dissolve. Cool and make the volume up to the mark with diluting solution. Filter it through 0.45 μm filter paper. From the above filtrate discard first few ml of filtrate and use remaining filtrate for analysis. Hence, the concentration of perindopril erbumine is 32.0 and of indapamide 10.0 μg/mL.

RESULTS AND DISCUSSION

In vitro dissolution study

The *in vitro* dissolution data showed the release was found to be 90-95% at the end of 45-60 min and by changing the concentration of polymer the release was less retarded. *In vitro* drug release data are shown in Figure 3.

Validation of the HPLC method

The HPLC method used to analyze the dissolution samples was validated according to current ICH and FDA guidelines. The validation included spe-

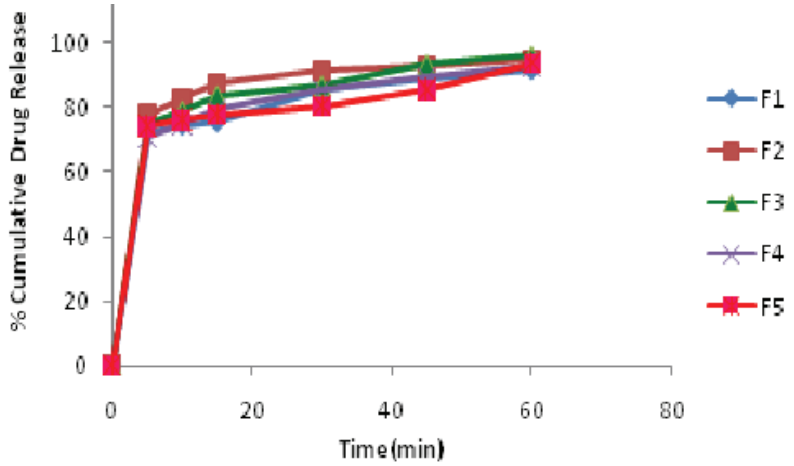

Figure 3. In vitro dissolution study: F1 (drug: polymer (1:1)), F2 (drug: polymer (1:1.5)), F3 (drug: polymer (1:2)), F4 (drug: polymer (1:2.5)), F5 (drug: polymer (1:3)).

cificity, linearity, accuracy, precision, range, robustness and solution stability studies.

Specificity

The specificity of the method was evaluated by injecting an aliquot of the dissolution medium (*i.e.*, deionized water) and the following: 1) a solution containing the APIs at nominal concentration, 2) a placebo solution prepared from a synthetic blend of the tablet excipients and 3) a sample solution prepared from a synthetic blend of the APIs and tablet excipients as lactose, dicalcium phosphate, hydroxylpropylmethyl cellulose, starch, polyvinyl pyrrolidone. These solutions were prepared in deionized water and sonicated at 37 °C for 20 min prior to being injected into the chromatographic system. As shown in Figure 4, there was no system, filter or excipients-related peaks that interfered with the quantitation of either active ingredient. These results demonstrate the specificity of the method.

System precision

The system precision of the method was evaluated by performing six replicate injections of a sample at the nominal PE (2.0/4.0) and indapamide 0.625/ /1.25 mg tablets (4 μg/mL PE and 1.25 μg/mL indapamide) standard concentrations. The sample was a synthetic blend of drug and excipients. The peak area *RSD* (%) of PE was 0.81% and indapamide was 1.61% which was considered acceptable.

Method precision

The *RSD* (%) of the sample response factor was calculated for six separate preparations at the nomi-

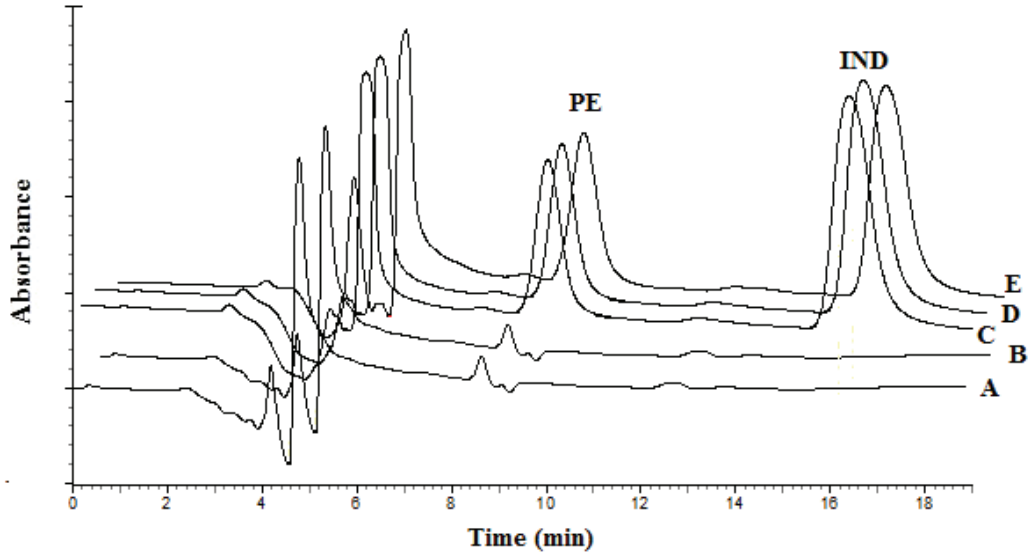

Figure 4. Representative of specificity chromatograms: A = blank, B = placebo, C = standard, D = standard + placebo, E = sample, PE = perindopril erbumin and IND = indapamide.

nal standard concentration of the PE 2.0/4.0 and indapamide 0.625/1.25 mg tablets (4 μg/mL PE and 1.25 μg/mL indapamide). The sample was a synthetic blend of drug and excipients. The peak area *RSD* (%) of PE was 1.26% and indapamide was 0.99%, which was considered acceptable for these low doses drug product formulations.

Intermediate precision

The intermediate precision of the method was determined by six replicate analysis of sample, by two different analyst using two different instruments, different columns, on different days at the nominal standard concentration of the PE 2.0/4.0 and indapamide 0.625/1.25 mg tablets (4 μg/mL PE and 1.25 μg/mL indapamide). Fresh sample and standard solutions were independently prepared on each day of analysis. The peak area *RSD* (%) of PE was 1.28% and indapamide was 1.03%, which was considered acceptable for drug product formulations.

Range and linearity

The calibration range in each medium was established by considering the practical range necessary for dissolution or assay, and to give accurate and precise results with good linearity. Detector response (area of peak) was plotted against concentration to obtain calibration curves. For PE and indapamide tablets, concentration range 70–130% drug concentration of PE (2.8, 3.2, 3.6, 4.0, 4.4, 4.8 and 5.2 μg/mL) and indapamide (0.88, 1.00, 1.13, 1.25, 1.38, 1.50 and 1.63 μg/ml) drug solution in pH 6.8 phosphate buffer and diluting solution was used. Regression analysis was carried out on calibration curves and results are summarized in Table 1. Linearity of the calibration curves shown in Figure 5 and the adherence to Beer's law were validated by the high value of the correlation coefficient.

Table 1. Calculated linear regression parameters

Parameter	Perindopril erbumine	Indapamide
r^2	0.995	0.996
Slope	36760	28188
y-Intercept	3337	-93152
Linearity equation	36760*x* + 3337	3722*x* - 38824

Accuracy

The accuracy of the method was evaluated at 80, 100 and 120% of the nominal assay concentration for PE, and at 80, 100 and 120% of nominal for indapamide. As indicated in Tables 2 and 3, the average recoveries ranged from 99.6 to 101.3% for PE and from 97.0 to 100.5% for indapamide. The accuracy of the method was considered acceptable based on its intended use.

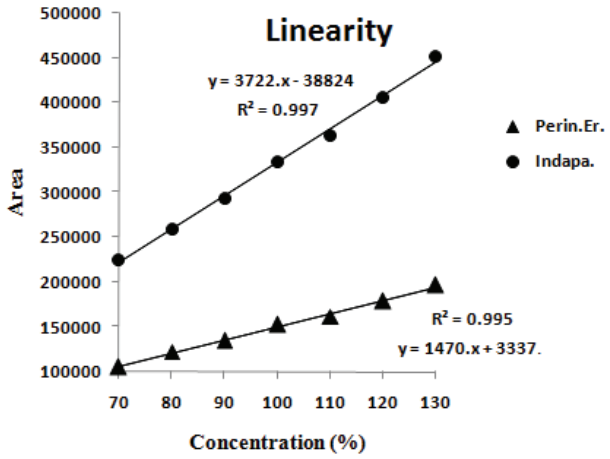

Figure 5. Linearity curves of perindopril erbumine and indapamide.

Table 2. Accuracy results for perindopril erbumine (recovery, %)

Sample	Nominal concentration, %		
	80	100	120
1	100.27	100.96	98.06
2	100.70	101.38	98.06
3	100.22	100.92	98.07
4	100.49	100.72	98.65
5	101.04	100.52	98.38
6	100.32	100.49	98.15
Average	100.51	100.83	98.23
RSD, %	0.31	0.33	0.24

Table 3. Accuracy results for indapamide (recovery, %)

Sample	Nominal concentration, %		
	80	100	120
1	100.64	98.96	99.51
2	99.72	98.38	99.68
3	99.32	98.51	99.50
4	98.49	98.84	99.25
5	98.74	98.24	98.88
6	98.37	99.19	99.15
Average	99.21	98.69	99.33
RSD, %	0.87	0.37	0.29

Robustness

The robustness of the method was evaluated during development by making small, but deliberate, changes to the method parameters. The experimental results of the ruggedness study are summarized in Table 4. Critical chemical and instrumental chromatographic parameters such as the composition and flow

Table 4. Ruggedness study of the HPLC assay

Chromatographic parameter	*RSD* (NMT 2), %	
	Perindopril erbumine	Indapamide
Optimal conditions	0.81	1.16
Variation of the mobile phase flow rate (±10%)		
Q = 0.45 ml min^{-1}	1.34	1.02
Q = 0.55 ml min^{-1}	1.19	0.99
Variation of the ACN:buffer ratio (±2 % ACN)		
39.2:60.8 (v/v)	1.58	1.70
40.8:59.2 (v/v)	1.08	1.58
Variation of column temperature (±5 °C)		
T = 45 °C	1.32	1.28
T = 55 °C	1.16	1.43
Variation of the wavelength (±5 nm)		
220 nm	1.65	0.97
230 nm	1.39	0.74

rate of the mobile phase, column temperature and wavelength were deliberately varied in the range of to their optimal values. These results demonstrate that the method is robust to small deviations from the nominal conditions.

Solution stability

The combined standard solution of PE and indapamide was stored at two different conditions (room temperature and 8 °C), unprotected from light, at ambient conditions and assayed after initial, 4, 8, 12, 16, 20 and 24 h against a initial prepared standard solution. All of the assay results during this time period were within 98-102% of the initial value and % response from initial is NMT 2 therefore no degradation products were observed in any of the chromatograms. The standard solution is therefore considered stable for at least 24 h under normal laboratory conditions and 8 °C conditions (Tables 5 and 6). Therefore, the method was supposed to be stability-indicating.

Table 5. Results for stability of analytical solution for perindopril erbumine

Time, h	Stability at room temperature		Stability at 8 °C	
	Area	Response from initial, %	Area	Response from initial, %
Initial (0)	165828	0	165828	0
4	165702	0.08	165376	0.27
8	165376	0.27	165848	-0.01
12	162278	2.14	164162	1.00
16	159498	3.82	163840	1.20
20	158650	4.33	160118	3.44
24	156168	5.83	159061	4.08

Table 6. Results for stability of analytical solution for indapamide

Time, h	Stability at room temperature		Stability at 8 °C	
	Area	Response from initial, %	Area	Response from initial, %
Initial (0)	304925	0	304925	0
4	307740	-0.92	302679	0.74
8	302679	0.74	303894	0.34
12	304843	0.03	304380	0.18
16	304380	0.18	308006	-1.01
20	304759	0.05	300104	1.58
24	299058	1.92	317699	-4.19

CONCLUSIONS

The developed HPLC technique is precise, specific, accurate and stability indicating. The developed method was validated based on ICH guidelines. Statistical analysis proves that the method is repeatable and selective for the analysis of perindopril erbumine and indapamide as bulk drug and in pharmaceutical formulations. The method can be used to determine the purity of the drug available from the various sources by detecting the related impurities. It may be extended to study the degradation kinetics of perindopril erbumine and indapamide and for its estimation in plasma and other biological fluids.

Acknowledgements

Authors would like to thank Principal, R.C. Patel Institute of Pharmaceutical Education and Research, Shirpur and Mepro pharmaceuticals Pvt. Ltd., for providing facilities to carry out this work.

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NAUČNI RAD

VALIDACIJA TESTA RASTVARANJA AKTIVNIH SUPSTANCI PERINDOPRIL ERBUMINA I INDAPAMIDA IZ KOMBINOVANE TABLETNE FORMULACIJE POMOĆU RP-HPLC METODE

Validacija testa brzine rastvaranja aktivnih supstanci perindopril erbumina i indapamida iz kombinovane tabletne formulacije je izvršena pomoću RP-HPLC metode. Testirana je usaglašenost sa globalnim regulatornim zahtevima. Validacija uključuje testiranje specifičnosti, linearnosti, tačnosti, preciznosti, opsega koncentracije, robustnosti i studiju stabilnosti. Brzina rastvaranja aktivnih supstanci je testirana u USP aparaturi i sa korpom koja se rotira brzinom 100 o/min u 1000 ml fosfatnog pufera pH 6,8 kao medijumu za rastvaranje. Rastvorene supstance su analizirane RP- HPLC metodom na 4.6 mm×250 mm×5µm cijano koloni. Temperatura kolone je 50 °C. Kao mobilna faza korišćena je smeša acetonitrilo: pufer pH 2.8::40:60 (v/v). Komponente su detektovane na 225 nm. Nađeno je da je testirana metoda selektivna, tačna i precizna u testiranom opsegu koncentracija. Varijabilnost metode u toku jednog i više dana je <2% RSD. Ova metoda je uspešno primenjena za kvantitativno određivanje perindopril erbumina i indapamida iz kombinovane tabletne formulacije.

Ključne reči: Perindopril erbumin; Indapamid; RP-HPLC; rastvaranje; validacija.