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Development of a RP-HPLC Method for Evaluating Losartan Potassium and Hydrochlorthiazide Tablets

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A simple, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed for the simultaneous determination of losartan potassium and hydrochlorthiazide in tablet dosage forms. A Lichrospher 100 C-18, 5 μm column having 20×4.6 mm i.d. in isocratic mode, with mobile phase containing 20 mM KH_2PO_4 buffer (pH 3): acetonitrile:tetrahydrofuran in 60:30:10 were used. The flow rate was 1.0 ml/min and effluent was monitored at 215 nm. The retention time of losartan potassium and hydrochlorthiazide were 7.94 min and 3.26 min, respectively. Linearity for losartan potassium and hydrochlorthiazide were in the range of 4-40 μg/ml and 1-10 μg/ml, respectively. Average percentage recoveries obtained for losartan and hydrochlorothiazide were 100.2 % and 100.1 %, respectively. *In vitro* evaluation of tablets containing losartan and hydrochlorothiazide was performed by evaluating tablets for thickness, diameter, hardness, tensile strength, disintegration and dissolution. Parameters for dissolution testing (dissolution medium and speed) were optimized. Dissolution testing was performed at 100 rpm in 0.1 N HCl as dissolution medium by paddle method. The proposed method is accurate, precise, specific and rapid for simultaneous estimation of losartan potassium and hydrochlorthiazide in tablets as well as for dissolution testing.

Losartan potassium (LOP) is an angiotensin II receptor (AT1) antagonist that acts by blocking the action of angiotensin II of renin-angiotensin-aldosterone system[1]. Chemically the drug is 2-n-butyl-4-chloro-1{[2'-(1H tetrazol-5yl)(1,1'-biphenyl)-4-yl] methyl}-1H immidazole-5-methanol. Several methods such as HPLC[2-4], spectrophotometric[5, 6] have been reported in literature to estimate losartan potassium alone in market formulations. Hydrochlorthiazide, chemically, 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide-1,1-dioxide[7]. It is official in IP, BP and USP. The literature describes HPLC[8-10], spectrophotometric[11] and non-aqueous potentiometric titration[12] methods for the analysis of hydrochlorthiazide. Fix dose combination containing losartan potassium and hydrochlorthiazide is available only in tablet dosage form in market. RP-HPLC[13] method and spectrophotometric methods[14,15] were reported for simultaneous estimation. The present report describes a precise, accurate, specific and sensitive RP-HPLC method for simultaneous estimation of losartan potassium and hydrochlorthiazide in tablets as well as for dissolution testing.

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MATERIALS AND METHODS

High performance liquid chromatograph including a Hitachi pump L-7110 equipped with universal injector 77251 (Rheodyne) with injection volume 20 μl, Hitachi L-7420 UV/Vis detector, Merck-Hitachi HSM software, Lichrospher 100 C-18, 5 μm column having 20×4.6 mm i.d. was used. Reference standard of LOP and hydrochlorthiazide were obtained form Torrent Pharmaceutical Ltd., Ahmedabad. Tablets having combination of LOP (50 mg) and hydrochlorthiazide (12.5 mg) were purchased from local pharmacy. Acetonitrile, water and tetrahydrofuran used

were of HPLC grade. Orthophosphoric acid and KH_2PO_4 of analytical reagent grade were used.

Preparation of mobile phase and standard stock solution:

Mobile phase was prepared by mixing 600 ml of 20 mM KH_2PO_4 (pH adjusted to 3.00±0.1 with 10 % v/v o-phosphoric acid) with 300 ml of acetonitrile and 100 ml of tetrahydrofuran. LOP (50 mg) and hydrochlorthiazide (12.5 mg) were dissolved in acetonitrile, ultrasonicated and diluted by mobile phase in such a way to get concentrations in a range of 4-40 µg/ml and 1-10 µg/ml, respectively. The diluted solutions were filtered through 0.45 µm cellulose nitrate filter. Sample solution was stable for 12 h in mobile phase.

Preparation of sample solution:

Twenty tablets were weighed accurately and finely powdered. Tablet powder equivalent to 50 mg LOP or 12.5 mg hydrochlorthiazide was taken in 50 ml volumetric flask, dissolved in 15 ml of acetonitrile and kept in an ultrasonic bath for 10 min. Further dilution was done with mobile phase up to the mark. The solution was filtered through 0.45 µm cellulose nitrate membrane filter paper. One ml of aliquot was diluted to 50 ml with mobile phase.

Chromatography:

The optimum mobile phase containing 20 mM KH_2PO_4, acetonitrile, tetrahydrofuran (60:30:10) was selected because it was found to ideally resolve the peaks of LOP (RT=7.94) and hydrochlorthiazide (RT=3.26), respectively as shown in fig. 1. Wavelength was selected by scanning standard solutions of both drugs over 200 nm to 400 nm wavelengths. Both components show reasonably good response at 215 nm.

Calibration and precision of the assay:

Aliquots of standard LOP and hydrochlorthiazide stock solution were taken in different 50 ml volumetric flasks and diluted up to the mark with mobile phase in such a way that the final concentrations of LOP and hydrochlorthiazide were in the range of 4-40 µg/ml and 1-10 µg/ml, respectively. Evaluation of two drugs was performed with UV/vis detector at 215 nm. Peak areas were recorded for all the peaks. The plots of peak area verses the respective concentration of LOP and hydrochlorthiazide were found to be linear in the range of 4-40 µg/ml and 1-10 µg/ml with co-efficient of correlation (r) 0.9999 and 0.9998, respectively. Working standard and sample solutions (n=5) were injected in to the universal injector 77251 (Rheodyne) with injection volume 20 µl. From the peak area of LOP and hydrochlorthiazide (Table 1), the amounts of drugs in samples (n=5) were computed.

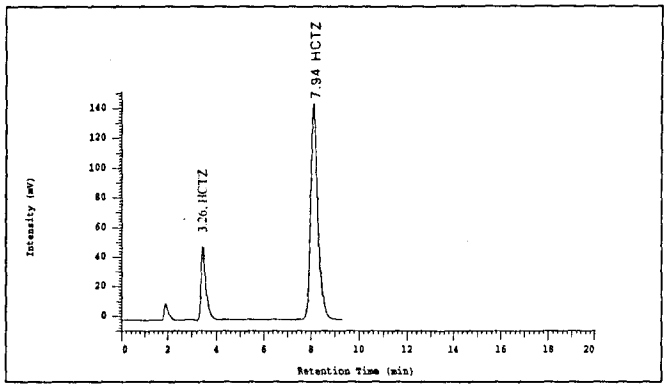

Fig. 1: Typical chromatogram of losartan potassium and hydrochlorthiazide

***In vitro* evaluation of tablets:**

In vitro evaluation of tablets containing LOP and hydrochlorthiazide was performed for thickness, diameter, hardness (diametric crushing strength), tensile strength, disintegration and dissolution. Diametric crushing strength was measured using Monsanto hardness tester (Shital Scientific Industry, Mumbai). Disintegration test was carried out using disintegration apparatus (model ED2, Electrolab, Mumbai) using 0.1 N HCl as a disintegrating media at 37±2°. Parameters for dissolution testing (dissolution medium and speed) were optimized using water and 0.1 N HCl as dissolution media at 50 rpm as well as 100 rpm using USP apparatus 2. Three different branded tablets containing LOP and hydrochlorthiazide were taken for dissolution testing. Dissolution study of tablets was carried out in a 900 ml of 0.1 N HCl, maintained at 37±0.5 ° at a speed of 100 RPM. Five ml of sample was withdrawn at time intervals of 15, 30, 45 and 60 min. The volume of dissolution fluid was adjusted to 900 ml by replacing each 5 ml aliquot withdrawn with 5 ml of 0.1 N HCl. The concentrations of losartan potassium and hydrochlorthiazide in the samples were determined by proposed RP-HPLC method.

RESULTS AND DISCUSSION

Analysis of tablets containing losartan potassium and hydrochlorthiazide was carried out by using optimized mobile phase containing 20 mM KH_2PO_4, acetonitrile, tetrahydrofuran (60:30:10) and detection was done at 215 nm. The average content of losartan potassium and hydrochlorthiazide found were 50.04 mg/tab (RSD=0.451 %) and 12.45 mg/tab

TABLE 1: LINEARITY DATA OF LOP AND HYDROCHLORTHIAZIDE.

Drug	Concentration	Peak area (mean ± SD)	RSD
HCTZ*	1	184852±1429.83	0.774
	2	253192.6±2366.55	0.935
	3	320425.2±1366.32	0.426
	4	392067.2±2088.77	0.533
	5	457080.4±4040.40	0.884
	6	536228.6±4553.12	0.849
	8	678370.4±3669.26	0.541
	10	797952.2±4832.89	0.606
LOP*	4	821254.0±4714.0	0.574
	8	1223484.4±10851.4	0.887
	12	1611443.8±9714.1	0.603
	16	1999403.3±12514.1	0.626
	20	2387362.7±15371.7	0.644
	24	2775322.1±18259.8	0.658
	32	3551241.0±30512.4	0.859
	40	4327159.9±29940.0	0.692

HCTZ – Hydrochlorthiazide and LOP – Losartan potassium

(RSD=0.809 %), respectively. The results obtained by proposed method were closed to the label claim of both the drugs (Table 2). The lower values of RSD in Table 1 indicate that the method is precise and accurate. As per USP XXIII, system suitability tests were carried out on freshly prepared standard stock solution of LOP and hydrochlorthiazide and parameters obtained with 20 µl injection volume were summarized in Table 2. The mean percentage recoveries of LOP (100.2 %) and hydrochlorthiazide (100.1 %) reveal no interference of excipients like starch, microcrystalline cellulose, talc or lactose in tablets (Table 2). The low COV values of intra-day and inter-day variations (Table 3) revealed that proposed method is robust and rugged.

Limit of detection (LOD) and Limit of quantification (LOQ) were found to be 0.08 µg/ml, 2 µg/ml and 0.1 µg/ml, 1 µg/ml of losartan potassium and hydrochlorthiazide respectively (Table 2). LOD and LOQ show that the method is sensitive to losartan potassium and hydrochlorthiazide. Three different brands (brand I, brand II, brand III) of tablets were taken for *in vitro* evaluation. Thickness, diameter and hardness (diametric crushing strength) were varied from brand to brand (Table 4). Therefore, it is difficult to compare the crushing strength of tablets of different size and shape. In present work tensile strength was used to compare the force necessary to break the tablets per unit area. The tensile strength of tablets was measured using Eqn. 1, which is $Ts = 0.0624 \times P/D \times T$, where P is the tablet crushing strength (Kg), D is the diameter (cm) and T was the thickness (cm).

Disintegration test on tables of three brands was per-

TABLE 2: VALIDATION AND SYSTEM SUITABILITY PARAMETERS

Parameter	HCTZ	LOP
Retention time (min)	3.26	7.94
Tailing factor	1.86	1.42
Theoretical plates	3812	6532
Calibration range (µg/ml)	1 – 10	4 - 40
% Recovery*	100.1±0.17	100.2±0.095
Amount of drug found (mg/tab)*	12.45±0.101	50.04±0.225
Resolution factor	-	11
Limit of detection (µg/ml)	0.1	0.08
Limit of quantification (µg/ml)	1	2 µg/ml

*Average of five determinations (average±SD)

TABLE 3 : INTRA DAY AND INTER DAY VARIATION (N = 3)

	Concentration (µg/ml)	Intra day variation (Mean Area ± SD)	Inter day variation (Mean Area ± SD)	COV	
				Intra day	Inter day
LOP	4	842274.7±8790.1	835661.0±11136.0	1.044	1.333
	16	2032339.0±22683.5	2056946.0±37786.8	1.116	1.837
	40	3572820.0±21973.1	3579105.0±36699.1	0.615	1.025
HCTZ	1	180898.0±1569.4	187181.0±2420.3	0.868	1.293
	4	392109.3±2527.7	393136.3±7460.1	0.645	1.898
	10	794200.7±4568.2	794337.7±9456.7	0.575	1.191

formed using 0.1 N HCl as a disintegrating media at 37±2°. Brand II have minimum disintegration time and due to this, % release at 15 min of brand II was relatively higher than brand I and brand III. Water and 0.1 N HCl were tried as dissolution medium and experiments were done at 50 RPM as well as 100 RPM using paddle method. In water, almost 85 % release of both drugs were observed at 50 RPM as well as 100 RPM paddle speed (Table 5). *In vivo,* film coated tablet formulations have to release drugs in acidic medium. Therefore attempts were made to do the dissolution testing in 0.1 N HCl. At 50 RPM, in 0.1 N HCl, % release of HCTZ was around 60 % (Table 5). Percentage release in 0.1 N HCl of hydrochlorthiazide was taken as the stringent limit to select optimized dissolution method, owing to comparative lesser solubility of hydrochlorthiazide than losartan potassium in 0.1 N HCl. Hydrochlorthiazide and many of its combinations are official in IP, BP, USP. By referring various monographs stated IP and USP, it was observed that stringent dissolution limit for HCTZ in 0.1 N HCl was 85 % of drug release of HCTZ at 30 min. In present work, 85 % release of HCTZ in 0.1 N HCl in 30 min was observed only at 100 RPM paddle speed (Table 6). While 85 % release of LOP was observed with almost all dissolution conditions. Therefore, dissolution testing was performed at 100 RPM in 0.1 N HCl as dissolution medium by paddle method and contents were analyzed by proposed RP-HPLC method. The proposed RP-HPLC method is accurate, precise, sensitive, selective and rapid for simultaneous estimation of losartan potassium and hydrochlorthiazide in tablet dosage form. It can be conveniently adopted for dissolution testing.

TABLE 4: SUMMARY OF *IN VITRO* EVALUATION OF VARIOUS PARAMETERS FOR TABLETS CONTAINING LOP AND HCTZ.

Parameters		Brand I	Brand II	Brand III
Thickness (mm)*		3.47	4.02	3.63
Diameter (mm) *		7.48	10.06	8.09
Hardness (Kp) *		5.90	10.20	7.30
Tensile strength –Ts*		0.0145	0.0160	0.0158
Disintegration (min)`		7.01	4.12	7.46
Dissolution at 30 min (% release) '◆	LOP	86.4	86.7	86.2
	HCTZ	86.7	87.1	86.4

*Average of three determinations. *Average of five determinations. ◆Dissolution testing was done in 0.1 N HCl at 100 RPM. Brand I - REPACE-H of SUN Pharmaceutical Ltd. (LOP 50 mg + HCTZ 12.5 mg) Brand II-TOZAAR-H of Cipla Laboratories. (LOP 50 mg + HCTZ 12.5 mg) and Brand III-ZAART-H of Emcure Pharmaceutical Ltd. (LOP 50 mg + HCTZ 12.5 mg)

TABLE 5: AVERAGE % DRUG RELEASE IN WATER AND 0.1 N HCL

Dissolution Medium	Speed (RPM)	Time (min)	Average % release of drugs					
			LOP			HCTZ		
			Brand I	Brand II	Brand III	Brand I	Brand II	Brand III
Water	50	15	65.0	71.0	67.3	49.0	61.6	44.6
		30	86.2	86.9	87.5	85.6	85.9	85.2
		45	90.4	88.8	88.2	87.8	87.8	87.8
		60	94.1	96.1	96.9	91.4	91.8	91.4
	100	15	69.3	74.7	72.9	65.1	70.7	62.5
		30	87.9	88.6	86.8	86.7	87.1	86.4
		45	93.2	94.1	92.3	91.2	92.4	90.4
		60	97.0	98.5	97.1	93.0	94.4	93.1
0.1N HCl	50	15	63.1	68.8	63.4	30.4	35.4	31.2
		30	81.4	85.6	82.8	58.5	65.7	60.2
		45	88.1	89.1	88.3	77.9	82.3	81.5
		60	89.9	92.3	90.1	88.2	88.2	87.5
	100	15	63.4	77.4	64.3	60.7	70.7	61.6
		30	86.4	86.7	86.2	86.3	87.2	86.5
		45	90.7	92.2	90.7	91.3	92.7	89.1
		60	92.0	93.4	93.0	93.2	94.2	93.2

TABLE 6: COMPARATIVE *IN VITRO* % DRUG RELEASE AFTER 30 MIN OF HCTZ AND LOP FROM MARKETED TABLETS

Dissolution medium	Speed (RPM)	Average % drug release at 30 min					
		LOP			HCTZ		
		Brand I	Brand II	Brand III	Brand I	Brand II	Brand III
Water	50	86.2	86.9	87.5	85.6	85.9	85.2
	100	87.9	88.6	86.8	86.7	87.1	86.4
0.1 N HCl	50	81.4	85.6	82.8	58.5	65.7	60.2
	100	86.4	86.7	86.2	86.3	87.2	86.5

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