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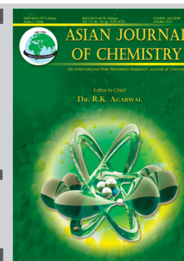

HPLC Analysis of Urapidil in Pharmaceutical Dosage Form

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A stability indicating HPLC method was developed for the determination of urapidil in pharmaceutical dosage form in presence of the degradation products. The chromatographic separation was achieved on an Inertsil ODS, 4.6 mm × 250 mm, 5 μm, column. The mobile phase contained a mixture of acetonitrile: 50 mM ammonium dihydrogen phosphate: triethanolamine (25: 75: 0.5, v/v, pH adjusted to 5.5 with orthophosphoric acid). The flow rate was 1.0 mL min^{-1} and the detection wavelength was 270 nm. The method was validated for the parameters like specificity, linearity, precision, accuracy, LOD, LOQ and robustness. It shows an excellent linearity over range of 10 to 160 μg/mL (r^2 = 0.9997). The mean recovery of urapidil ranged from 99.16 to 100.04 % while intra and interday relative standard deviations were < 2.0 %. The method could be applied for stability testing as well as routine quality control analysis of urapidil in bulk drug and pharmaceutical formulation.

Key Words: Column liquid chromatography, Stability indicating study, Estimation, Urapidil.

INTRODUCTION

Urapidil (6-{3-[4-(*o*-methoxyphenyl)-l-piperazinyl] propylamino}-1,3-dimethyluracil (Fig. 1) has been shown to be an effective, safe and well-tolerated drug for the treatment of hypertension, management of perioperative hypertensive crisis in the hospital, as well as in some cases of medical life threatening hypertension[1-3]. Its action is mainly due to a postsynaptic α-receptor antagonism that inhibits the vasoconstrictive action of catecholamines and reduces blood pressure by decreasing peripheral vascular resistance. It has an agonistic effect on central 5-HT1A receptors and lowers blood pressure by inhibiting the pressor baroreceptor[4,5]. Urapidil treatment also improves coronary flow, myocardial perfusion and left ventricular function following PCI in patients with ST-elevation ACS[6]. The majority of adverse events occurring during urapidil therapy are mild and transient, usually subsiding after long term treatment. The most common events reported during oral or intravenous therapy are dizziness, nausea and headache. Adverse events associated with intravenous urapidil are usually due to a rapid decrease in blood pressure[7,8].

Since urapidil is a widely used drug, an effective method for its analysis is highly desirable. Literature survey reveals that few methods have been published for analysis of urapidil in the bulk form and in pharmaceutical formulation. Current methods include HPLC[9-11], flow injection-chemiluminescence[12-13], fluorescence spectrophotometry[14] and voltammetric[15]. The disadvantages of these methods include low sensitivity, long analysis time, and unreported solution stability data. The purpose of this study was to develop an accurate, rapid and reproducible method for analysis of urapidil in the presence of degradation products. The proposed method will be helpful to the pharmaceutical industry for day-to-day analysis of urapidil in quality control as well as stability study of bulk material and in pharmaceutical dosage forms.

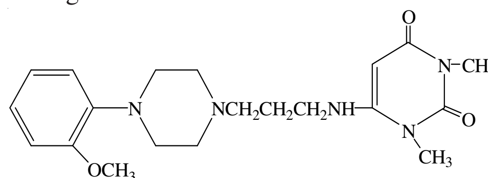


Fig. 1. The chemical structures of urapidl

EXPERIMENTAL

Urapidil bulk drug was obtained as a gift sample from Fuhe-Huaxing Pharmaceutical Group Co., Ltd. (Zhaodong, China) and Lixiding® tablets (contain urapidil 30 mg per tablet) were obtained from the Rejoy Pharmaceutical Co., Ltd. (Xi'an, China). Standard sample of urapidil was supplied by NICPBP (Beijing, China). HPLC-grade acetonitrile were received from Dikma Technology Inc. (Richmond, USA). Analytical grade ammonium dihydrogen phosphate, orthophosphoric acid and triethanolamine were purchased from Concord Technology Co. Inc. (Tianjin, China). Analytical grade sodium hydroxide, hydrogen peroxide and hydrochloric acid were purchased from Kermel Chemical Reagent Co., Ltd. (Tianjin, China).

Chromatography: HPLC was performed with a Beckman (USA) 125 pump, 168 DAD detector and a Rheodyne model 7725i injection valve with 20 µL sample loop. The compounds were separated on a 4.6 mm × 250 mm, 5 µm particle, Inertsil ODS analytical column with acetonitrile: 50 mM ammonium dihydrogen phosphate: triethanolamine 25: 75: 0.5 (v/v, pH adjusted to 5.5 with orthophosphoric acid) as isocratic mobile phase at a flow rate of 1 mL min^{-1}. The injection volume was 20 µL and the 270 nm was selected as the detection wavelength. The chromatographic signals were monitored and integrated by use of Beckman Gold Chrom software.

Preparation of standard solution: Twenty mg amount of urapidil was weighed and dissolved in a 50 mL volumetric flask with acetonitrile to yield 400 µg/mL stock solution. The standard working solutions of urapidil in the concentration range 10-160 µg/mL were prepared by diluting the stock solution with mobile phase. All the stock and working solutions were stored at 4 ºC and brought to room temperature before use.

Preparation of sample solution: Twenty tablets each containing 30 mg urapidil were accurately weighed and crushed to a fine powder. An accurately weighed portion of the powder equivalent to 20 mg of urapidil was transferred to a 50 mL volumetric flask containing 30 mL of acetonitrile and sonicated for 0.5 h. The final volume made up to 50 mL with acetonitrile, resulting mixture was filtered through 0.22 µm filter. This solution was further diluted by mobile phase to achieve 40 µg/mL of urapidil.

Validation of the method: The method was validated for specificity-forced degradation studies, calibration linearity and range, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) and robustness.

System suitability: This test has been performed in order to make the complete testing system suitable for the intended application. The system suitability was assessed by five replicate analyses of urapidil at the concentration of 40 µg/mL. The acceptance criteria were not more than 2 % for the RSD of the peak areas and retention times, not more than 1.2 for the tailing factor of the peak of urapidil.

Specificity-forced degradation studies: The ability of an analytical method to unequivocally assess the analyte in the presence of other components (impurities and degradation products) can be demonstrated by evaluating specificity. The specificity of the method was determined by injecting impurity reference samples along with urapidil. The impurity reference samples usually generate from force degradation by acid hydrolysis, base hydrolysis, oxidation, light exposure and heat.

For acid hydrolysis, base hydrolysis and oxidation, urapidil (8 mg) was separately dissolved in 10 mL of 1 M HCl, 1M of NaOH and 3 % H_2O_2, all the mixtures were reflux at 80 ºC for 1 h. For heat degradation sample was exposed to heat at 100 ºC for 48 h and for light degradation, the sample was exposed to sunlight lamp (5000 ± 500lx) for 120 h. Samples were withdrawn at appropriate times, acid and base treated samples were neutralized and then all the five treated samples were diluted by mobile phase to get final concentration of 40 µg/mL. All the solutions were filtered through 0.22 µm filter and subjected to HPLC by the method described above to detect peaks of degradation products.

Calibration and linearity: Calibration curve were constructed in the range of 10-160 µg/mL for urapidil to encompass the expected concentration in measured samples. Curve was obtained by plotting the peak area *versus* concentration of the drug and treated by least-squares linear regression analysis.

Limit of detection and limit of quantification: The limits of detection and quantification were determined by the signal-to-noise (S/N) ratio for urapidil by analyzing a series of diluted solutions until the S/N ratio was about 3 for LOD and 10 for LOQ, respectively.

Precision: The precision of the method was assessed by study of repeatability and intermediate precision. Repeatability (intraday) of the assay method was evaluated by six injections of three concentration levels of urapidil (20, 40 and 80 µg/mL) in one day and the values of relative standard deviation were calculated to determine intraday precision. Intermediate precision (interday) at the same concentrations was determined on successive days.

Accuracy: To assess the method's accuracy, the recovery was evaluated by adding known amount of the drugs with three concentration levels (80, 100 and 120 % of original amount determined in urapidil samples) into the urapidil samples. The contents were reanalyzed by the proposed method and percentages of recovery were calculated.

Robustness: To determine the robustness of the developed method, the chromatographic conditions flow rate (± 0.2 mL), acetonitrile content (± 2 %) in mobile phase and column oven temperature (± 2 ºC) were deliberately altered respectively and degradative solution of urapidil in oxidative condition was injected under each condition. The effect of these changes on resolutions between urapidil and its closest degradation product, tailing factors and RSD of peak areas of urapidil were evaluated.

RESULTS AND DISCUSSION

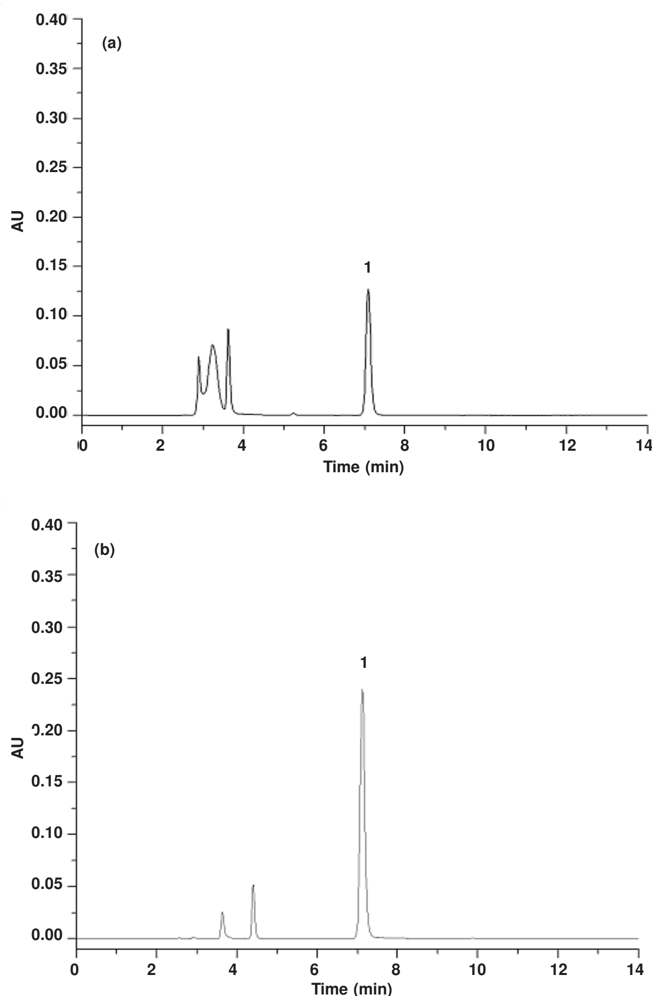
HPLC method development and optimization: Selection of the HPLC conditions were guided by the requirement for good resolution of adjacent degradation product peaks within as short time as possible, especially when large numbers of samples were analyzed. Method development was started with a ODS column and a series of aqueous mobile phases containing ammonium dihydrogen phosphate solution and different volume fractions methanol or acetonitrile as organic phase in different pH. Satisfactory separation was achieved with acetonitrile: 50 mM ammonium dihydrogen phosphate 25:75 (v/v, pH adjusted to 5.5 with orthophosphoric acid), but urapidil peak was asymmetry and a little tailing. So triethanolamine as modifier was added into mobile phase, urapidil peak became symmetrical. Finally, it was found that the quality separation in terms of peak symmetry, resolution, reasonable run time and other factors were obtained by use of acetonitrile: 50 mM ammonium dihydrogen phosphate: triethanolamine 25: 75: 0.5 (v/v, pH adjusted to 5.5 with orthophosphoric acid) as mobile phase and Inertsil ODS column. The optimum flow rate, determined by testing the effect of flow rate on retention time and resolution, was 1.0 mL min^{-1}. All experiments were performed at ambient temperature. In this method, wavelength 270 nm was selected so that there will be no interference from excipients and solvents and maximum absorbance for urapidil.

System Suitability: The system suitability test solution was injected and the chromatographic parameters like relative standard deviation of the peak area and retention time for replicate injections of urapidil and tailing factor for peak were evaluated. The relative standard deviation of peak area and retention time for replicate injections of urapidil was 0.82 and 0.53 %, respectively. The tailing factor for urapidil peaks was 1.06. This indicates the suitability of this system.

Specificity-forced degradation studies: The specificity of the developed method is illustrated in Fig. 2. During the forced degradation study, degradation was not observed in urapidil stressed samples that were subjected to light and heat. However, the degraded products were observed under oxidative condition, base hydrolysis and acid hydrolysis. The retention time of urapidil is *ca.* 7.10 min and no interfering peak was found at this retention time. Therefore, this indicates that the method is specific and stability indicating.

Calibration and linearity: The calibration was based on the analyses of the calibration standard solutions of urapidil at six concentration levels ranging from 10-160 µg/mL. The corresponding linear regression equation was A = 11243C + 5231 with square of correlation coefficient (r^2) of 0.9997. The results show that an excellent correlation existed between the peak area and concentration of the analyte.

Limit of detection and limit of quantification: Under the developed HPLC conditions. The limit of detection (LOD)



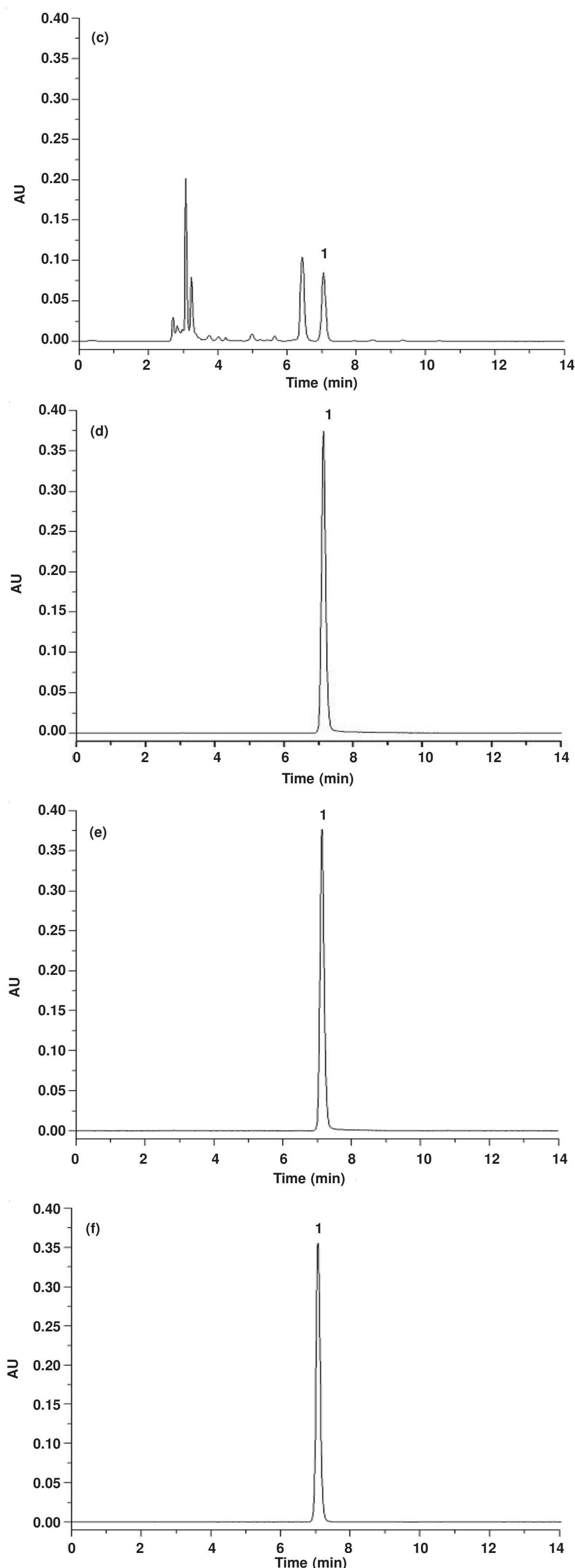


Fig. 2. LC chromatograms of urapidil (a) After acidic degradation (b) After basic degradation (c) After oxidative degradation (d) After heat degradation (e) After light degradation (f) Standard solution (40 µg/mL).

and limit of quantification (LOQ) for urapidil were found to be 0.033 and 0.10 μg/mL, respectively.

Precision : In Table-1 the results related to intraassay and interassay variability obtained from the assay of the standard solution, are reported. The RSDs for intraday precision ranged from 0.664-1.130 % while for interday precision they were 0.940-1.220 %. They are well within the limit of 2.0 % confirming high precision of the method.

TABLE-1
INTRA-DAY AND INTER-DAY PRECISION OF URAPIDIL (n=6)

Actual concentration (μg/mL)	Intra-day precision		Inter-day precision	
	Concentration measured (μg/mL) mean ± S.D.	RSD (%)	Concentration measured (μg/mL) mean ± S.D.	RSD (%)
20	20.331 ± 0.173	0.852	20.605 ± 0.194	0.940
40	40.434 ± 0.268	0.664	40.813 ± 0.446	1.093
80	78.773 ± 0.891	1.130	78.062 ± 0.953	1.220

Accuracy: The mean recovery of urapidil ranged from 99.16 to 100.04 % at three different concentrations (Table-2). Excellent recoveries were made at each added concentration. It was confirmed from the results that the developed method is highly reliable and consistent.

TABLE-2
RECOVERY STUDY OF URAPIDIL FROM SAMPLES WITH KNOWN CONCENTRATIONS

Concentration levels (%)	Added Amount (μg/mL)	Recovered Amount (μg/mL)	Recovery (%)	Mean recovery (%)
80	32.00	32.19	100.60	99.80
	32.00	32.01	100.02	
	32.00	31.61	98.78	
100	40.00	40.31	100.77	100.04
	40.00	39.52	98.79	
	40.00	40.22	100.55	
120	48.00	48.16	100.33	99.16
	48.00	47.61	99.18	
	48.00	47.02	97.95	

Robustness: The elution order and the resolution between the closest eluted peak corresponding to urapidil were not significantly affected. Results about the robustness study are depicted in Table-3. RSDs of peak areas and tailing factors of the urapidil in all robustness parameters were examined and found to be well within the limit of 2 % and 1.2. These indicate the method is robustness and provide consistent and reliable results, which are not affected by small changes in experimental conditions.

Tablet application: The drug content in the tablet formulation was quantified using the proposed analytical method. The commercially available Lixiding® tablets were assayed. The mean content (n = 6) for urapidil was 99.56 % and the percentage RSD value for the six assay values was 1.039 %, which confirms the method is suitable for routine analysis of the compound in pharmaceutical preparations.

TABLE-3
ROBUSTNESS PARAMETERS OF HPLC METHOD

Factor	Level	Results		
		Resolution (R)	Tailing (T)	Peak area RSD (%)
Mobile phase composition (acetonitrile: 50 mM ammonium dihydrogen phosphate: triethanolamine, v/v)	73:27:0.5	1.82	1.12	0.92
	75:25:0.5	1.86	1.06	
	77:23:0.5	1.79	1.05	
Flow rate of mobile phase (mL/min)	0.8	1.88	1.02	1.18
	1.0	1.86	1.06	
	1.2	1.62	1.09	
Column temperature (°C)	23	1.75	1.02	0.86
	25	1.86	1.06	
	27	1.87	1.03	

Conclusion

A simple specific stability indicating liquid chromatographic method with isocratic elution was developed for the quantification of urapidil in a tablet formulation. This method was validated and found to be accurate, precise, repeatable, linear, specific and selective for the detection and quantification of urapidil. The retention time is relatively short for urapidil, *i.e.* 7.10 min, which enables rapid quantitation of many samples in routine and quality control analysis of the tablet formulation. The same solvent was used through out the experimental work and no interference from any excipient matrix was observed. The method could therefore find be practical application for stability testing as well as routine quality control analysis of urapidil in bulk drug and pharmaceutical formulations.

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