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Pharmaceutical Sciences **REVIEW ARTICLE……!!!**

A REVIEW ARTICLE ON ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF CERTAIN ANTIHYPERTENSTIVE DRUG BY RP-UPLC METHOD

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KEYWORDS:

Method Development, Ultra pressure liquid chromatography, Column temperature, validation parameters, stress study.

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ABSTRACT

The main requirement of pharmaceutical company is to reduce the fair in the development of new drugs to enhance sensitivity, specificity, robustness, resolution for their detection. This can be achieved by UPLC which is the modified HPLC method comprising high pressure and small sized particles (less than 2µm) used in the column. The review article reveals the method development and validation of stability indicating on certain antihypertensive drugs by RP- UPLC. The different mobile phases like acetic acid, triethylamine, acetonitrile, phosphoric acid, methanol, ortho phosphoric acid, trifluoroacetic acid, and ammonium acetate, potassium dihydrogen phosphaste buffer were used in RP-UPLC. The chromatographic separation was carried by different stationary phases like C18, Dionex C18, Acquity UPLC BEH C18, Kromasil Eternity TM C 18, Acquity HSS-T3, Zorbax XDB-C18,Acquity BEH phenyl,ODS column which are processed by using isocratic method The validation method was evaluated with various parameters like linearity, precision, accuracy, robustness, LOD and LOQ. The evaluation of stability of drugs was performed with various degradation parameters such as oxidation, hydrolysis, acid base hydrolysis and photolytic hydrolysis. From the review, it was observed that C18 column was efficient for the separation due to the smaller particles size provides better resolution and retention time. The different mobile phases like like Ortho phosphoric acid, acetonitrile, phosphoric acid, methanol, triethyamine, trifluoroacetic acid and ammonium acetate were generally favors for the effective separation and yields good retention time. The developed method by RP-UPLC was validated per the ICH guidelines and found to be specific, precise, accurate and linear. Hence, the review was clearly indicated that UPLC method is possible to develop a new sensitive and accurate for different pharmaceutical formulations.

INTRODUCTION:

Analytical procedures play a critical role in equivalence and risk assessment, management. It helps in establishment of product specific acceptance criteria and stability of results. The Validation should demonstrate that the analytical procedure is suitable for its intended purpose.[1] Analytical method development and validation are the continuous and inter-dependent task associated with the research and development, quality control and quality assurance departments.[1] Ultra performance liquid chromatography is a modern technique which gives a new direction for liquid chromatography which enhance mainly in three areas like speed, resolution and sensitivity.[1] Ultra pressure liquid chromatography will make the most of the separation performance (by reducing dead volumes) and according to the pressures about 8000 to 15,000 PSI compared with 2500 to 5000 PSI in HPLC[2]. UPLC applicable for particle less than 2µm in diameter to acquire better resolution, speed, and sensitivity compared with high-performance liquid chromatography (HPLC)[1] . Due to the use of fine particle the column length can be reduced then automatically it will give more efficiency in result. The column temperature maintained in the ultra- give the peak area results.[2]. Different detectors like optical tunable ultraviolet, fluorescence, refractive index, light scattering and mass spectrometric are used in UPLC. Due to the very sharp and narrow peaks there are more number of peaks appear in short time which might facilitate in analysis of complex mixtures and which may give more information regarding the samples should be analyzed [2]. The separation and quantification in UPLC are done under very high pressure (up to 100M Pa). The UPLC was validated in terms of specificity, precision, robustness, reproducibility, and linearity. These are validated with respective to limits.[3]

Different pharmaceutical formulations were analyzed by RP UPLC method:

Different pharmaceutical formulations were analyzed by RP UPLC method and summarized Ch Krishnaiah et al [4] has developed and validated a novel and rapid RP-UPLC method for the estimation of Valsartan in pharmaceutical dosage form in the presence of its impurities and degradation product. The chromatographic column acquity UPLC BEH C-18 100mm, 2.1mm, 1.7 µm particle size is used. sovlent A-mixture of 1.0% acetic acid and acetonitrile(90:10 v/v), solvent B-mixture of 1.0% acetic acid and acetonitrile(10:90 v/v). The flow rate of mobile phase was 0.3ml/min. The UPLC gradient program was set as 0.01/20, 1.0/40, 3.5/55, 6.5/80, 8.5/80, 8.9/20, 9.5/20. The column temperature maintained at 27°C and detected at wavelength 225nm. The injection volume was 1.0 µL. stress condition of UV light(254nm), heat(60°C), acid(2N HCl at 60°C), base(0.05N NaOH at 60°C), hydrolytic(60°C) and oxidation(6.0%H2O2 at 60°C).For heat and light studies , study period was 10 days ,for hydrolytic, base it was 24 hrs; acid 1 hrs and oxidation it was 5 hrs. The method was linear for valsartan and the linear regression value obtained

was >0.9979, precision was validated by intra-day and interday assays. Recovery data were in the range 98.3%to 101.5% with R.S.D. values < 1.1% .The short retention time of 0.27ml/ min. The developed method was validated according to the ICH guidelines with respect to linearity, precision, accuracy, specificity and robustness.

T.Kaleemullah et al [5] a simple reverse phase ultra performance liquid chromatography (RP-UPLC) method for the quantitative determination of 2-(4',4'-Dibromomethylphenyl)benzonitrile impurity content at low level in Irbesartan drug substance. Separation was achieved with 100mm x 2.1mm, 1.7µm particle size, ODS column. The mobile was a gradient prepared by simple phosphoric acid buffer, and Acetonitirle at a flow rate of 0.1 mL min-1, UV detection was performed at 205nm. The method was validated to confirm selectivity, precision, linearity and accuracy parameters as per ICH guideline. the detection limit and quantification limit are found to be 0.41 µg ml-1 and 1.46 µg ml-1 respectively. Repeatability is good, with a relative standard deviation of 2.1% to 3.2%.Recovery data was 99.3%with R.S.D.value 2.2 %.The linear regression value was 0.99956. stress condition study for Thermal (dry heat at 105°C for 120 hrs.), photolytic condition (white fluorescent light (10K Lux/120 hrs followed by UV light 200 watt-hours/m20) humidity condition (80% RH at 25°C for 120hrs.), oxidative condition: (30% H2O2 solution and exposed to 85°C for 60 min.), hydrolytic condition: (sample solution was mixed with purified water and kept aside for 12h.)

S.Raj et al[6] This method was developed for the quantitative determination of olmesartan medoxomil (OLM) in active pharmaceutical ingredient (API) and pharmaceutical dosage forms. Chromatographic separation was achieved on Acquity UPLC BEH phenyl 100mm, 2.1mm, and 1.7lm phenyl columns and the gradient eluted within a short run time, that is, within 10.0min. The eluted compounds were monitored at 210nm, the flow rate was 0.3mL/ min and the column oven temperature was maintained at 27°C. The high correlation coefficient (R2>0.9991), R.S.D value was within 1.0% , intra and interday repeatability are within 2.0% , recovery range from 98.5% to 101.8% , The drug was subjected to stress study neutral hydrolysis (60°C 8hr); basic (0.001N NaOH at 60°C 4hr); oxidative degradation (1.0% H2O2 at 60°C 30min), acidic hydrolysis (2N HCl at 60°C 1hr) . the method was validated according to the present ICH guide lines for specificity, limit of detection, limit of quantification, linearity, accuracy, precision, ruggedness, and robustness.

Sirisha [7] A validated ultra performance liquid chromatography (UPLC) method has been developed and validated for the simultaneous determination of losartan potassium and

chlorthalidone in pharmaceutical preparations. Chromatographic separation was achieved by injecting a volume of 0.5µl of standard into HSS C18, (100 mm x 2.1x 1.8 µm) column .The mobile phase of composition 560 mL of solution A (1.36g of potassium dihydrogen phosphate buffer of pH 3.0) and 440ml mL of solution B (acetonitrile and methanol in 9:1 ratio) was allowed to flow through the column at a flow rate of 0.4ml per minute for a period of 3.0 min. Detection carried out at a wavelength of 230 nm. The retention time were found to be 0.72 and 1.89min for losartan potassium and chlorthalidone respectively. Good linearity was observed over the concentration range of 12.5 to 125 µg/ mL for losartan and 3.125 to 31.25 µg/ mL for chlorthalidone with correlation coefficient > 0.999 for both the drugs. The % recovery was ranged from 99.56% to 100.03% for losartan potassium and 98.73% to 100.34% for chlorthalidone. the method was validated according to the present ICH guide lines for specificity, limit of detection, limit of quantification, linearity, accuracy, precision, ruggedness, and robustness.

Reddy et al[8] A novel, rapid, specific and stable RP-UPLC-MS assay and its Organic Impurities method was developed and validated for the estimation of Trandolapril in Active pharmaceutical ingredient. The separation was carried out by using a mobile phase consisting of Solution A: 0.1% TFA in water, Solution B: 0.1% TFA in Acetonitrile. Solvent -Acetonitrile: water (2:8).The column used for separation was Column: Acquity UPLC BEH C18, 100 mm x 2.1 mm x1.7µm with flow rate of 0.4ml/min and wavelength at 210nm. The retention time for Trandolapril was 5.56. system suitability from assay standard was 0.22% , precision and intermediate precision for assay was 0.5%and 0.4%, linearity ranges from0.05 -25µg mL−1. The regression coefficient (R2) was 0.99998. Recovery (%) of Trandalopril ranged from 100 to 101.0% for samples. stress conditions of exposed to acid (0.1N HCl for 1 hour reflux at 80°C), base (0.1 N NaOH for 10 minutes at room temperature), oxidation (6% peroxide for 24 hours at room temperature), Thermal (Exposed at 105°C for 48 h) and photolytic stress (1.2 million lux hours followed by 200watthours per square meter). The method was also validated in terms of accuracy, precision, linearity, system suitability, robustness and ruggedness as per ICH guidelines.

R.K.Seshadri et al[9] A simple ultra performance liquid chromatographic (UPLC) method has been developed for the simultaneous estimation of Metoprolol (MT), Atorvastatin (AT) and Ramipril (RM) from capsule dosage form. The method was developed using Zorbax XDB-C18 (4.6 mm x 50 mm, 1.8 µm) column with a mobile phase consisting of 0.06% ortho phosphoric acid in Milli Q water having an ion pair reagent, 0.0045 M Sodium lauryl sulphate as buffer, at ratio of buffer: Acetonitrile (50:50 v/v), at 55°C column temperature with a flow rate of 1.0 ml/min. Detection was

carried out with ultra-violet detection at 210 nm for RM, MT and AT respectively. The retention times were about 1.3, 2.1 and 2.6 min for MT, AT and RM respectively. The % mean recoveries are 101.8, 102.1 and 101.4 for MT, AT and RM respectively. Regression coefficient was found to be R2 0.9996, 0.9998, 0.9995 for MT, AT, RM. The % RSD of assay was found to be less than 2% MT (0.4%), AT(0.3%), RM(0.8%). Degradation was not observed in visible light, UV, humidity and water hydrolysis stress studies. Significant degradation was not shown in acid hydrolysis, base hydrolysis and oxidative conditions. However, thermal stress showed significant degradation. The verification of peak purity indicates that there is no interference from degradants, facilitating error-free quantification of MT, AT and RM. Thus, the method is considered to be "Stability-indicating". The method was found to be rugged and robust and can be successfully used to determine the three drugs and its combinations. The method was developed and validated as per ICH guidelines.

Singhal M et al [10] Candesartan Cilexetil is an antihypertensive agent currently available in combination with Hydrochlorothiazide. A simple, rapid, reliable and robust reversed phase ultra-performance liquid Chromatography (RP-UPLC) method was developed as per International Conference on Harmonization (ICH) guidelines. The best separation was achieved in less than 5 minutes on a 50 × 2.1 mm, 2.2 µm particle size Dionex C18 column with the gradient mobile phase 5 mM, 6.2 ± 0.5 pH ammonium acetate buffer - acetonitrile at a flow rate of 0.5 mL/min at 215nm. Linearity ranges is 10-200ppm. Resolving time for chorthalidone (Rt = 4.34) and Candesartan Cilexetil (Rt = 4.86). correlation coefficient of the regression (r): Candesartan Cilexetil (0.998 ± 0.00075) chorthalidone(0.999 ± 0.00045) . The percentages of the recoveries obtained were 93.31 - 96.26 % and 100.53 - 102.43 %. LOD was 3.04 and 2.82. LOQ was 9.23 and 8.56. for Candesartan Cilexetil and Chlorthalidone respectively . regression coefficient (R2): Candesartan Cilexetil (0.999 ± 0.00047)) chorthalidone (0.999 ± 0.00028). Retension time was found to be 4.86 and 4.43 for Candesartan Cilexetil and chorthalidone. Resolution time is that 2.618. Assay of bulk drug is found to be 98.33 ± 1.30, RSD 1.30% and 101.60 ± 0.65 RSD 0.64% for Candesartan cillexetil and chlorthlidone respectively. the method was validated according to the present ICH guide lines for specificity, limit of detection, limit of quantification, linearity, accuracy, precision, ruggedness, and robustness.

Mallikarjuna et al [11] A stability indicating Ultra Performance Liquid Chromatography (UPLC) method was developed and validated for the simultaneous determination of Atorvastatin Calcium (ASC) and Amlodipine Besylate (AMB) in tablets. The chromatographic separation was performed on acquity UPLC, Kromasil C18, 50×2.1 mm, 3.5 µm using gradient elution of acetonitrile and

0.1% v/v Triethyl amine buffer (pH 3 ± 0.05) at flow rate of 0.8 ml/min. UV (Ultra Violet) detection was performed at 240 nm. Total run time was 2.2 min within which main compounds and degradants were separated. Column temperature at 40°C. Injection volume is 2 µL. Forced degradation studies condition - acidic (1N HCl, 60°C, 6 hours), alkaline (1N NaOH, 60°C, 60 min), strong oxidizing (3% H2O2, 60°C, 60 min), thermal (60°C, 48 hours) and photolytic (254 nm, 1 day). The LOD for amlodipine and atorvastatin was found to be 0.3 µg/ml and 0.2 µg/ml. The LOQ for amlodipine and atorvastatin was found to be 0.7 µg/ml and 0.6 µg/ml. correlation coefficient R2 > 0.9990. Recovery level is 100.46% for amlodipine , 99.64% for atorvastatin. The method was validated for accuracy, repeatability, reproducibility and robustness.Linearity, Limit of Quantification (LOQ) and Limit of Detection (LOD) was also established as per ICH guidelines.

Table 1: stability studies in pharmaceutical formulation by using RP- UPLC method

Sr. No.	Drug name	Uses	Mobile phase	Stationary phase	Parameter	References
1	Valsartan	Treatment of hypertension	Mixture of solvent A-1.0% acetic acid Buffer:acetonitrile(90:10 v/v) Solvent B-1.0% acetic acid buffer : acetonitrile(10:90v/v)	Aquity Uplc BHEC18 (100mm x 2.1mm, 1.7µm)	Linearity, accuracy, precision, specificity, robustness, LOD and LOQ	[4]
2	Irbesartan	Treatment of hypertension	Gradient prepared by simple phosphoric acid buffer and acetonitrile A-0.01%v/v ortho phosphoric acid + water ; B-acetonitrile	Aquity Uplc BHEC18 (100mm x 2.1mm, 1.7µm)	Linearity, accuracy, precision, specificity, robustness, LOD and LOQ	[5]
3	Olmesartan medoxomil	Treatment of hypertension	Mobile phase A-mixt of.buffer 10mm potassium dihydrogen phosphate pH 2.5 + acetonitrile (90:10 v/v)	Aquity Uplc BHE phenyl (100mm x	Linearity, accuracy, precision, specificity, robustness, LOD and LOQ	[6]

			, phase B-mixt of buffer + acetonitrile (20:80 v/v)	2.1mm, 1.7µm)		
4	Losartan potassium and chorthalidone	Treatment of hypertension	Mobile phase(mixt.A &B) in ratio 56:44 v/v ; A-potassium dihydrogen phosphate buffer pH 3.0 ; B-acetonitrile : methanol(90:10 v/v)	Aquity Uplc BHEC18 (100mm x 2.1mm, 1.8µm)	Linearity, accuracy, precision, specificity, LOD and LOQ	[7]
5	Trandolapril	To treat mild to moderate hypertension	Solution A-0.1%trifluoroacetic acid(TFA) in water. Solution B-0.1%TFA in acetonitrile ;solvent-acetonitrile:water(2:8)	Aquity Uplc BHEC18 (100mm x 2.1mm, 1.7µm)	Linearity, accuracy, precision, specificity, robustness, ruggedness,LOD and LOQ	[8]
6	Metoprolol, Atorvastatin, Ramipril	Treatment of hypertension	0.006% ortho phosphoric acid in milli –Q water as buffer 0.04m sodium lauryl sulfate buffer : acetonitrile (50:50 v/v)	Zorbax XDB C18 4.6mm x50mm x1.8µm	Linearity, accuracy, precision, specificity, robustness,ruggedness LOD and LOQ	[9]
7	Candesartan cilexetil and chorthalidone	Treatment of hypertension and chronic heart failure	gradient mobile phase 5 mM, 6.2 ± 0.5 pH ammonium acetate buffer - acetonitrile	Dionex C18 50mm x 2.1mm x 2.2 µm	Linearity, accuracy, precision, specificity, robustness, LOD and LOQ	[10]
8	Amlodipine besylate , Atorvastin calcium	Treatment of hypertension and high cholesterol	Gradient elution of acetonitrile +0.1% v/v trimethylamine buffer (pH 3+-0.05)	Acquity UPLC kromasil C18 50mm x 2.1mm x 3.5 µm	Linearity, accuracy, precision, specificity, robustness, LOD and LOQ	[11]

Conclusion:

It can be concluded that some important mobile phases like potassium di hydrogen, acetonitrile, sodium phosphate, methanol, sodium di hydrogen, trifluoro acetic acid and ammonium acetate buffer were commonly used for method development and validation of drugs by RP-UPLC method. In stationary phase, C18 column was efficient for the separation when comparison to that of other columns. The developed method by RP-UPLC was validated per the ICH guidelines and found to be specific, precise, accurate and linear. Hence, it was clearly indicated that UPLC method is possible to develop a new sensitive and accurate for different pharmaceutical formulations. It was fulfilled that the UPLC method was simple, precise & robust and can be useful for determination of purity of many drugs in API.

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