SUMMARY
OF
THE PRESENT RESEARCH
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Estimation of drug content and controlling the impurities in pharmaceutical product plays a important role to ensure quality, safety and efficacy of product. Various regulatory authorities and pharmacopeias have recognized the importance of this to the drug development process and have separately increased validation requirements in recent years.

During initial stage of drug development, the drug substance or drug product will be subjected to various stress conditions like light, temperature, acid, alkaline and humidity to know the stability of drug. As per regulatory requirement the product will be subjected for accelerated stability conditions to asses shelf life of product. In both the cases drug will undergo degradation and forms various degradants. Hence Stability indicating analytical methods are more essential for quantitative estimation of drug content and impurities in the samples.

Among the several instrumental techniques available for the assay and impurities of drugs, HPLC is a versatile tool for the qualitative and quantitative analysis of drugs and pharmaceuticals, chemical and biological materials. In recent years, Ultra-performance liquid chromatography (UPLC) has been investigated as an alternative to HPLC for the analysis of pharmaceutical compounds.

To address the above concerns, the author has verified the present state of development of analytical methods for some of the widely used drugs. The following eight drugs namely., Rabeprazole, Domeridone, Omeprazole, Telmisartan, Chlorthalidone, Metoprolol, Atorvastatin and Ramipril to whom there is a wide scope for
the development of new analytical methods for their assay and impurities determination. The author has developed novel methods for determination of assay and impurities by HPLC/UPLC for the drugs mentioned above.

The present research work has been divided into five chapters as described below and appropriate references have been mentioned at the end of each chapter.

Chapter -1 is divided into two sections. Section (i) describes the importance of stability indicating methods, requirements of stability testing and types of different analytical techniques employed in literature for developing a stability indicating methods. HPLC instrumentation and its importance in reverse phase chromatography.

Section (ii) describes the general methodology of method development and validation. In the present research, UPLC/HPLC with PDA and VWD detectors are used for method development and validation.

Chapter -2 Deals with the development and validation of stability indicating impurity method and identification and characterization of three potential degradation products of Rabeprazole. This chapter is further divided into two sections. Section (i) starts with the introduction giving a brief such as chemical name, structure, mode of action, characteristics and literature on physico chemical properties reported for Rabeprazole. Rabeprazole drug substance and drug product is official in Indian Pharmacopeia.
Section (ii) describes the experimental details of stability indicating impurity method by UPLC with UV detector. A simple, sensitive and reproducible ultra high performance liquid chromatography (UPLC) coupled with a photodiode array detector method is developed for the quantitative determination of Rabeprazole (RAB) and its three potential degradant impurities and six process impurities in pharmaceutical dosage forms. Three unknown impurities are formed in the drug product under the stress conditions [40°C/75% relative humidity (RH) for 6 Months] with relative retention times (RRT’s) 0.10, 0.18 and 0.31 on UPLC. A Thorough study was undertaken to characterize these potential degradants. These impurities are enriched by subjecting drug to various stressed conditions, isolated using preparative HPLC and characterized by NMR and Mass spectroscopy. On the basis of the spectral data, the Impurity-I, Impurity-II and Impurity-III are characterized as 2-Amino-1H-benzimidazole, 1H-Benzimidazol-2-ol and 2-Benzimidazolethiol. Chromatographic separation was achieved on Acquity BEH shield, RP18 column (100 x 2.1 mm i.d., 1.7 µm particle size), and the gradient eluted within 18 minutes runtime. The eluted compounds are monitored at 280nm, the flow rate is 0.4ml/min, and the column oven temperature is maintained at 25°C. The resolution between RAB and its nine impurities (process related and degradation) is greater than 1.5 for all critical components. The high correlation coefficient (r=0.999) values indicated clear correlations between the investigated compound concentrations and their peak areas within the test ranges. Method validation is performed as per International Conference on Harmonization (ICH) guidelines for specificity, limit of detection, limit of quantification, linearity, accuracy, precision, ruggedness and robustness.
Chapter -3 deals with development and validation of stability indicating assay method for simultaneous estimation of Omeprazole (OZ) and Domperidone (DP) from capsule formulation by RP-UPLC. A single gradient RP-LC method is developed for estimation of impurities in OZ and DP from capsule formulation. This chapter further divided into three sections.

Section (i) starts with introduction on application of combination product and providing a background of drug molecules such as chemical names, chemical structures, mode of action, literature on physico chemical properties. None of the reported articles described the single method for estimation of impurities and assay in the fixed dose combination product of Omeprazole +Domperidone capsules. Omeprazole drug substance is official in USP/EP/BP/IP, Domperidone drug substance is official in EP/BP/IP where as drug product of Omeprazole is official in UPS/BP/IP and Domeridone is official in BP/IP. But combination product is not official in any of the pharmacopeia. The novelty of research is single chromatographic method for assay of both the drugs and similarly single chromatographic method for estimation of both molecule impurities. The current method development includes forced degradation study under various stress conditions like acid hydrolysis, base hydrolysis, oxidation, heat and UV. Both analytical methods are validated as per International Conference on Harmonization.

Section (ii) describes the experimental details of assay method developed by reverse phase UPLC with UV detector. The method is developed using Acquity BEH Shield-RP18( 100x 2.1mm, 1.7µm) column with mobile phase consist of pH 6.4 phosphate buffer with 0.1% triethyl amine and acetonitrile in the ratio 50:50(v/v) as mobile phase A and 90:10 (v/v) as mobile phase B. The flow rate of mobile phase is 0.3ml/min.
temperature is 30 °C and detection wave length is 280 nm. The retention times are about 1.9 and 2.5 min for omeprazole and domperidone respectively. The run time is about 4 min. The gradient program is (Time/ %-B) 0/35, 1/40, 2/40, 2.5/35 and 4/35.

The method is validated as per ICH guidelines and found to be linear, accurate, precise, specific, robust and rugged. The method can be used for the determination of two drug components from combination dosage form.

Section (iii) describes the experimental details of impurities method. Five impurities of each OZ and DP compounds (process related and degradation impurities) are determined in single gradient stability indicating RP-HPLC method using UV detector. The Waters HPLC system with a diode array detector is used for method development and forced degradation studies. The HPLC system with UV detector is used for method validation. The method employs an Inertsil ODS 3V 250 x 4.6mm with 5 µm particles column with mobile phase consist of 0.05M monobasic potassium phosphate buffer pH adjusted to 7.2 using 0.1N sodium hydroxide solution and acetonitrile in the ratio of 75:25(v/v) as mobile Phase A and pH 7.2 phosphate buffer and acetonitrile in the ratio of 45:55 (v/v) as mobile Phase B. The flow rate of the mobile phase is 1.2 mL min\(^{-1}\). The column is maintained at 25 °C, the detection wavelength is 285 nm and gradient program Gradient program (Time/%B) : 0/0, 20/0, 30/75, 40/75, 50/0 and 55/0. is used for impurities detection of both compounds. The injection volume is 20 µL.

The HPLC method developed for impurities determination of Omeprazole and Domperidone is validated as per International Conference on Harmonization guidelines (ICH). The method validation shows satisfactory data for all the method validation
parameters tested. The developed method is stability indicating and can be used for assessing the impurities in OZ and DP capsules and also their individual dosage forms.

Chapter 4 deals with the development and validation of stability indicating assay and impurities methods by Reverse Phase Liquid Chromatography (RP-LC) for the Telmisartan (TS) and Chlorthalidone (CT) in its fixed dose combination product. This chapter is further divided into three sections.

Section (i) starts with the introduction giving a brief account such as chemical name, structure, mode of action, characteristics, commercially available formulations and literature on physicochemical properties reported for TS and CT. TS and CT drug substances are official in USP/EP/BP/IP where as Telmisartan drug product is official in USP/IP. Chlorthalidone drug product is official in USP/BP/IP. The combination product is not official in any of the pharmacopeias. So far to our current knowledge there is no method reported in any of the Pharmacopoeia or in the literature for the simultaneous determination of TS+CT in fixed dose drug product for assay and impurities.

Section (ii) describes the details of experimental procedures involved in stability indicating reversed phase UPLC method with UV detection. A Gradient RP-UPLC method for the stability indicating assay of TS and CT in its fixed dose combination product is developed. The method employs an Acquity BEH Shield (100 x 2.1 mm, 1.7 µ particle size) column. The buffer in the mobile phase consisting of 0.025M potassium dihydrogen phosphate, 0.0027M 1-hexane sulphonic acid sodium salt and 1ml of triethyl amine in milli-Q water (pH 4.5 ±0.05 adjusted with diluted ortho phosphoric acid. Mixture of pH 4.5 buffer and acetonitrile in the ratio 90:10 (v/v) is used as mobile phase-
A and in the ratio of 20:80 (v/v) is used as mobile phase-B. The flow rate of the mobile phase is 0.3 mL min\(^{-1}\). The column temperature is 25 °C and the detection wavelength is 235 nm. The injection volume is 3µL. Mixture of pH 4.5 buffer, acetonitrile and methanol in the ratio 60:20:20 (V/V/V) is used as diluent. The gradient program is (Time/%-B) 0/40, 1/95, 1.5/95, 2.5/40 and 3/40.

The method is validated as per International Conference on Harmonization (ICH) guidelines and found to be specific, precise, linear, rugged, robust and stability indicating. The method is suitable for the quantification of assay in TS and CT fixed dose combination products and also can be used for the individual drug product. Section (iii) describes the details of experimental procedures involved in stability indicating RP-UPLC method with UV detection for determination of one impurity of CT and seven degradation impurities of TS from fixed dose combination drug product.

The gradient UPLC method employs an Acquity BEH Shield (100 x 2.1 mm, 1.7 µ particle size) column. The buffer in the mobile phase consisting of 0.025M potassium dihydrogen phosphate, 0.0027M 1-hexane sulphonic acid sodium salt and 1ml of triethyl amine in milli-Q water (pH 4.5 ±0.05 adjusted with diluted ortho phosphoric acid). Mixture of pH 4.5 buffer and acetonitrile in the ratio 90:10 (v/v) is used as mobile phase-A and in the ratio 20:80 (v/v) is used as mobile phase-B. The flow rate of the mobile phase is 0.2 mL min\(^{-1}\). The column temperature is 25 °C and detection wavelength is 235 nm. The injection volume is 2µL. Mixture of pH 4.5 buffer, acetonitrile and methanol in the ratio of 60:20:20 (v/v/v) is used as diluent. The gradient program is (Time/%-B) 0/20, 2/30, 5/45, 8/55, 10/80, 14/80, 14.1/20 and 18/20.
The results of stress testing undertaken according to the International Conference on Harmonization (ICH) guidelines reveal that the method is selective and stability-indicating. The proposed method is accurate, precise, specific, and has the ability to separate all the potential impurities from each other, from actives and from excipients peaks found in the tablet dosage form. The method is suitable for the quantification of impurities in TS+CT fixed dose combination drug product and also their individual dosage forms.

Chapter -5 deals with the development and validation of stability indicating assay and impurities method. The assay method is developed using UPLC for the simultaneous estimation of Metoprolol (MT), Atorvastatin (AT) and Ramipril (RM) from capsule dosage form. Two separate reverse phase LC methods are developed by HPLC for estimation of impurities. Metoprolol impurities are determined in single method. Atorvastatin and Ramipril impurities are determined by another separate method. This chapter is further divided into three sections. Section (i) starts with introduction and briefing of three molecules characteristics like chemical name, structure, mode of action and literature on physico chemical properties. All three drug substance monographs are available in United States Pharmacopoeia (USP), British Pharmacopoeia (BP), Indian Pharmacopeia(IP) and European Pharmacopeia (EP) where as MT and RM drug products are official in USP, BP and IP and AT drug product is official in Indian Pharmacopoeia. So far to current knowledge there is no method reported--for simultaneous determination of MT+AT+RM in pharmaceutical formulation for assay and impurities.
Section (ii) describes the experimental details of stability indicating reversed phase UPLC method with UV detection. The method is developed using Zorbax XDB-C18 (4.6mm * 50mm, 1.8µm) column with a mobile phase consisting of 0.0045M sodium lauryl sulphate with 0.06% ortho phosphoric acid buffer and acetonitrile in the ratios of 50:50 (v/v). The column temperature is 55°C with a flow rate of 1.0 mL/min. Detection is carried out with UV at 210nm for RM, MT and AT respectively. The retention times are about 1.324, 2.148 and 2.684 min for MT, AT and RM respectively. The method is validated as per International Conference on Harmonizatio guidelines (ICH) and found to be linear, accurate, precise, specific. The method is found to be rugged and robust and can be successfully used to determine the three drugs and its combination dosage forms.

Section (iii) describes the details of experimental procedures involved in the development of stability indicating gradient RP-HPLC method with UV detection for determination of three MT impurities (degradation impurities), in another method two AT impurities (degradation impurities) & three RM impurities (degradation impurities). The first method employs an Vydac-C8 (150 x 4.6 mm, 5 µ particle size) column with a mobile phase of 0.01M potassium dihydrogen phosphate buffer (pH 3.0 ±0.05) as mobile phase-A, milli-Q water and acetonitrile in the ratio 10:90 (v/v) as mobile phase-B respectively. The flow rate of the mobile phase is 1.0 mLmin⁻¹. The column temperature is 40 °C and the detection wavelength of 223 nm (MT known and unknown impurities). The injection volume is 20µL. Methanol is used as diluent. The gradient programme is (Time/ %-B) 0/10, 40/50, 50/50, 51/10 and 60/10.
The second method employs an X-Terra RP18 (250 x 4.6 mm, 5 µ particle size) column with pH 5.2 buffer (3.0 g of Sodium Perchlorate and 1.0g of Disodium hydrogen phosphate in 1000 ml of milli Q water). Mobile phase A consists of buffer and acetonitrile in the ratio 80:20 (v/v), mobile phase B consists of buffer, acetonitrile and methanol in the ratio 20:70:10 (v/v/v). The flow rate of the mobile phase is 0.8 mLmin\(^{-1}\). The column temperature is 60 ºC and the detection wavelength of 210 nm (AT & RM known and unknown impurities). The injection volume is 20µL. Methanol is used as diluent. The gradient programme is (Time/ %-B) 0/0, 15/5, 25/15, 30/25, 35/30, 45/30, 65/35, 75/45, 90/40, 95/40, 100/30 and 120/0.

The results of stress testing undertaken according to the International Conference on Harmonization (ICH) guidelines reveal that the methods are selective and stability-indicating. The proposed methods are accurate, precise, specific, and has the ability to separate all the potential impurities from each other, from actives and from excipients peaks found in the tablet dosage forms. The methods are suitable for the quantification of impurities in MT+AT+RM fixed dose combination drug products.

The author is, thus successful in developing a precise, accurate, sensitive stability indicating assay and impurities methods for fixed dose combination products of Omeprazole+Domperidone capsules, Telmisartan+Chlorothalidone tablets and Metoprolol+Atorvastatin+Ramipril capsules by HPLC/UPCL. The author has identified, isolated and characterized three unknown degradation products in Rabeprazole tablets by using LC-MS and NMR spectroscopy and impurity method which is developed by UPLC is capable to separate nine process related and degradation products.