“SPECTROSCOPIC & CHROMATOGRAPHIC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF ANTIMICROBIAL AGENTS & THEIR COMBINATIONS IN SYNTHETIC MIXTURE AND ASSAY METHOD FOR THE PHARMACEUTICAL DOSAGE FORMS”

A
Thesis
Submitted to the
Atmiya University,
For the Degree
of

Doctor of Philosophy

in

PHARMACEUTICAL SCIENCES

by

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January, 2023

Summary

Introduction

The Anti-Microbial agents are those medicinal agents & drug substances that are utilized in the therapy for the infectious diseases caused due to the micro-organisms like bacteria, virus, fungus, protozoa, parasites, and other organisms that causes illness & health problems in the individuals. These medicinal agents are widely used in individual as well as in the combinational forms for the the preventions as well as for the therapy of diseases in the body. They are also referred as chemotherapeutic agents. For the newly approved drugs used in the therapies, individually as well as in the combinational therapies requires the quality control testing analytical methods for pure drugs as well as their pharmaceutical dosage forms.

The present research work comprises of the Stability Indicating RP-HPLC analytical method development and validation for selected drugs & pharmaceutical dosage forms belonging to the Antimicrobial class. The antimicrobial drugs are frequently upgraded and newly approved drugs by FDA and CDSCO are available in the markets in the form of new dosage forms as well as combined dosage forms with other drugs.

For the newly approved drugs and their drug combinations, the Stability analytical methods are not available in pharmacopoeia and even dissolution methods for the solid dosage forms are also not available for the combined dosage forms. Hence there is a need of newer analytical methods, as well as stability, indicating rapid- RP-HPLC technique and it is optimized for, analysis of drug during different stress conditions as well as in pure from and solid dosage form along with the *in-vitro* dissolution studies carried out for solid dosage forms.

Working Standard drugs- Doravirine, Lamivudine, Tenofovir, Cabotegravir, Rilpivirine, Dolutegravir, Fexinidazole, Maribavir, Molnupiravir, Amoxicillin, Clarithromycin, Vonoprazan, Combined formulations, Chromatographic HPLC system Shimadzu LC-20-AT, UV Spectrophotometer systronics & shimadzu-1800, Hypersil-coloumn ODS-C_{18} (250 mm x 4.6 mm, 5 µm id), Analytical balance, sonicator, Dissolution apparatus Veego Microprocessor. The Stability Indicating RP-

SUMMARY

HPLC methods for selected antimicrobial drugs have been developed & validated using ICH recommendations, which demonstrate its accuracy, precision & robustness of the procedures.

The ICH parameters Accuracy, Precision, LOD, LOQ, Linearity, Range, Selectivity were successfully performed within the criteria specified by ICH guidelines. The dergadants and pure drugs were successfully identified by the RP-HPLC approach in the stability and forced-degradation investigations under various stress conditions. This approach is used to investigate the *in vitro* dissolution characteristics of drug in solid oral dosage forms. The developed analytical method are validated as per the regulatory ICH guidelines and thus are accurate, precise and reliable for the utilisations in the testing of the drug substances and drug products.

The developed method experimentally practically can should be effortless to validate, it should, have been to be developed along with the major goal's with the goal-aims to rapidly test into the different-types of pre-clinical samples that are adopted, formulation pro-totypes-pre-formulations, different types of pharmaceutical formulations as well as in commercial marketed samples.

Chapter 1- Introduction

The Analytical Method Development is an procedure in which the different methods for the testing and analysis are developed discovered invented ,that are been made in majorly as in for the lab-industrial-testing & analysis drug substances active pure api-pharmaceutical ingredients in pure form and in the different types of pharmaceutical dosage forms.

The analytical method development is a procedure for generating different methodologies, processes, protocols, materials, protocols, generated for the testing analysis measurement of the analytes- i.e. drugs, api, solutes, impurities, excipients, preservatives and additives in pharmaceutical drug substances.

The Validation of the Analytical method procedures is the course of action in which it justifies the practical ability of a analytical method which meets the necessities to perform the laboratory analytical studies that can be applied for the purpose for which it has been produced.

The Identification & quantification of drugs , medicine, API ,impurities is an important crucial-critica—important processes-task in pharmaceutical process development for quality-regulations and safety-regulations purposes is made efective. The incidence-occurance-presence of, these unnecessary-undesired foreign substances chemicals-products., even in small quantity, amounts may influence-degenerate-deteriorate's, in the drug-medicinal agents-efficacy and safety-patient compliance's for of the pharmaceutical products, and hence need for into the criteria for generating., Various analytical methodologies-techniques-procedures., are utilized, employed-made,in the for the determination, analysis of major drugs-related components in Newly-approved-pharmaceutical dosage forms. There is a great-immense and severe, -need for development of newer analytical methods for analysis-testing's & validation for quality evaluation-assesment-estimation's-testings., of new emerging drugs.

It includes-involves the plans made for definitions, Background-information's data-gathering, drug profile, review literature, Selection of samples and analytes. Selection of the suitable method for the analytes on the basis of the physical, chemical nature of chemical substance and its pharmaceutical dosage forms. Specific procedure, tests & pretreatments for samples if required.

SUMMARY

Review of methods of analysis is required for, if present for the chemical substance, Laboratory Lab-analytical- method development-process, it includes., involves the generation of the various stages namely- sample preparation standards-samples of the drugs, specific analytical method, extraction & isolation, identification, purification, Instrument selection, optimization, detection, data-processing-analysing-testing of data, Generation of the major testing procedure's.

The developed method experimentally practically can should be effortless to validate, it should, have been to be developed along with the major goal's with the goal-aims to rapidly test into the different-types of pre-clinical samples that are adopted, formulation pro-totypes-pre-formulations, different types of pharmaceutical formulations as well as in commercial marketed samples.

The importance for the method development-procedures-methologies and the validation is for in assuring the quality of the product, as along with the well as in the Analysis of Active drug-medicinal's, molecules substances & Drug product, also it is very much essential for the recently approved drug Molecules, Phyto-chemical and can be done in the herbal phyto-chemical products

Method development is applied in the Impurity profiling, Residual microanalysis, Component of interest in matrices in the dosage forms.

The method development validations are applied in the to achieve, the criteria's for the- the acceptance products in accordance guided by international agencies, it's also a one of the important deliberately performed, mandatory requirement for registration of any pharmaceutical product, Validation of the analytical procedures not only it would improve the processes-procedures-methods, but also confirms that, processes- procedures adopted are properly-accurately been generated and developed, those which are efficient in analysis, as well as it deepens the perceptive of process and reduces the risk's in the, troubles, It decreases-reduces., the risk of defect-impurities- costs,- It decreases-reduces, removes errors's into the risk of regulatory-noncompliance, A effusive-whole totally validated processes., may require less-decreased level's in-process controls- ipqc and end product –final-quality,testing, Related-substances, drug, -products-impurities, related-products, by-products, -impurities., that are the, undesired-foreign substances, unwanted- that present or remain-hang about- along-with the active ap--pharmaceutical drug products., that has been or may might been during develop along with the substances, during short or long-term, stability testing, or can might be

generated autonomously into the, develop during preparations-compunding-formulation or upon long term storage-aging duration of both API-pure-drug-forms, and of for the formulated-APIs into the to medicines, The Validation of the developed analytical methods processes are a significant criteria which supports the method to be, accordance with the parameters of precise and accurate for the purpose of testing-quality-control and the analysis.

It includes validation parameters carried out per in accordance criteria of ICH guideline Q2-R1 like-Accuracy, Precision, Repeatability, Intraday precision, Interday Precision, Linearity, Range, -Specificity, Recovery, Ruggedness, Robustness, LOD,.-Limit of Detection., and the other one is the LOQ:-Limit of Quantification.

It is most essential for the New Dosage Forms- detecting the instability of API, Incompatibility with other active and inactives, The studies are performed for the elucidation intrinsic, -inherented,- stability distinctiveness of different the of drug substances. Majorly, these types of the, testing-analysis are one of the is part of the development-plan's approaches and strategy and therefore it is essentially carried performed made out conceded normally together to be analysed approaches- carried out as in under into the strong-more severe stress forced degradations conditions, that are been adopted into the usable purposes as in those used along in the for accelerated tests. The stress testing is performed and conducted to mainly provide data on forced decomposition of the drug substances, pure forms and drug- products and decomposition as well as degradations of for the drug substance. The guideline Q1A (R2) Stability Testing Process of New Drug Substances and Products.

Chapter 2 – Aims And Objectives

The combinational antimicrobial drugs for therapy contains two or more drug moiety which are to be utilized for the treatment requires quick reliable analysis method.

Analysis & Testing of these combinational drugs are difficult or erroneous i.e. error inducing at the time of simultaneous analysis in various dosage forms in laboratories by the chemist or analyst. Hence it is of prime importance for the testing of drugs by a safe, quality, efficient , reliable and validated analytical methods for the drugs in synthetic mixture as well as in marketed formulations

In the Selected class of antimicrobial agents there is necessity of Analytical method Development and Validation for the testing of the therapeutic drug combinations as per the following points that indicates major problem domains and areas which creates a necessity for the proposed research work. The newer antiviral drugs requires an appropriate validated method for qualitative and quantitative analysis.

The Main Research objective is Analytical Method development & Validation by adopting spectroscopic & chromatographic techniques for the selected class of Antimicrobial Agents & their combinations in pure api synthetic mixture and assay method for the pharmaceutical dosage forms.

The specific objective is for research is that, the selected drug combinations for which the analytical methods have been developed and validated as per ICH guidelines:

1. HPLC METHOD DEVELOPMENT AND VALIDATION FOR DELSTRIGO COMBINATION
2. STABILITY INDICATING HPLC DEVELOPMENT AND VALIDATION FOR DELSTRIGO COMBINATION
3. HPLC METHOD DEVELOPMENT AND VALIDATION FOR CABOTEGRAVIR AND RILPIVIRINE
4. STABILITY INDICATING HPLC METHOD DEVELOPMENT AND VALIDATION FOR CABOTEGRAVIR AND RILPIVIRINE
5. HPLC METHOD DEVELOPMENT AND VALIDATION FOR DOLUTEGRAVIR, TENOFOVIR AND RILPIVIRINE
6. STABILITY HPLC METHOD DEVELOPMENT AND VALIDATION FOR DOLUTEGRAVIR, TENOFOVIR AND RILPIVIRINE

7. HPLC METHOD DEVELOPMENT AND VALIDATION FOR FEXINIDAZOLE
8. STABILITY INDICATING HPLC METHOD DEVELOPMENT AND VALIDATION FOR FEXINIDAZOLE
9. HPLC METHOD DEVELOPMENT AND VALIDATION FOR MARIBAVIR
10. STABILITY INDICATING HPLC METHOD DEVELOPMENT AND VALIDATION FOR MARIBAVIR
11. HPLC METHOD DEVELOPMENT AND VALIDATION FOR MOLNUPIRAVIR
12. STABILITY INDICATING HPLC METHOD DEVELOPMENT AND VALIDATION FOR MOLNUPIRAVIR
13. HPLC METHOD DEVELOPMENT AND VALIDATION FOR VOQUEZNA COMBINATION- AMOXICILLIN, CLARITHROMYCIN AND VONOPRAZAN
14. STABLITY HPLC METHOD DEVELOPMENT AND VALIDATION FOR VOQUEZNA COMBINATION- AMOXICILLIN, CLARITHROMYCIN AND VONOPRAZAN

To produce cost effective, economic & easy analytical method for particular the selected class of drugs, dosage forms, in synthetic mixture as well as in marketed formulationsThe significance to perform this Research is to bring about newer methods of analysis for newly approved drugs (API/Dosage Forms) which are required for testing in Quality Control & Assurance departments in Industry, Analytical Laboratories, Academics or in any field of pharmacy/ analytical chemistry. Application of the Developed method for the formulations in synthetic mixture as well as in marketed formulations. As new/novel drugs are approved & their various dosage forms are constantly upgraded , which do not have any official analytical techniques, for that economic, reliable and practically applicable analytical methods must be generated.

Chapter 3 – Review of Literature

The The literatures have been reviewed for the analytical methods found for the selected class of drugs. The Review of the literatures have been done for the triple drug combination of Doravirine, Lamivudine, Tenofovir. These drugs belong to the class of NNRTI Non Nucleoside Reverse Transcriptase Inhibitors-class and that been used into therapy to the HIV virus infections in the adults and juvenile patients. These drugs are used individually as well as in the combinations with the other class of antiviral drugs for the therapy of HIV AIDS in the patients. There are reported methods for these drugs in different formulations with other class of drugs, UV, spectrophotometric, RP-HPLC , LCMS and other methods are reported. But none of them includes stability indicating RP-HPLC- method, for triple drugs Doravirine, and- Tenofovir, Lamivudine together- combined tablet dosage forms along with the application in the *in-vitro* dissolution applicable by the developed HPLC method.

For the combination of Cabotegravir & Rilpivirine the literature reviews have been done, they belong to NNRTI Nucleoside-Reverse-Transcriptase-Inhibitors- drug-class that are been applied-used in HIV infections, for which LCMS, UV Spectroscopic, HPLC methods have been present for the individual drugs as well as with the other drug combinations, but there are no reported methods for the stability indicating assay method by RP-HPLC applied-made for the injectable dosage form of the Cabotegravir & Rilpivirine along within combined dosage forms.

Review of the literature has been done for the dugs Dolutegravir, Rilpivirine & Tenofovir combinations which are used in the HIV type-1 virus infections as well as in hepatitis virus infections. These drugs are available in tablet oral dosage forms with different drug combinations. There are reported LC/MS/MS, UV spectrophotometric, HPLC , method for individual and with other drug combinations, but no reported stability indicating HPLC method in combinational-combined dosages available for them. Hence there is need for the analytical method for these drugs in combined-forms.

SUMMARY

Fexinidazole drug is a nitro-imidazole derivative used as a Anti-trypanosomal class of drug, used in HAT disease known as HAT Human African Typanosomiasis sleeping sickness which is induced by *Trypanosoma brucei gambiense* organism. Its *in vivo* metabolites are helpful in treatment of infectious disease induced by *Trypanosoma brucei* and *Leishmania donovani*. The literature review [91-95] there is RP-HPLC method for tablet dosage form. For this drug, no stability indicating RP-HPLC - method as well as in-vitro dissolution applicable HPLC method for this drug.

Molnupiravir is a is a drug which applied for the therapy of COVID-19 infections due to SARS COV 2 virus. The drug is effectively used in the therapy for patients in which new mutated viruses are made in active through inhibiting viral replication due to miss-interpretation in replication as drug is a nucleoside analogue of cytidine. The drug Molnupiravir is a isopropyl ester of hydroxyl cytidine and its acts by blocking RNA Polymerase to stop viral replication. The drug is approved therapy in viral infections. Literature reviews show RP-HPLC method as well as micellar UV spectrophotometric method in plasma as well as with combination with ritonavir darunavir, HP-TLC method is available but no in-vitro dissolution applicable RP-HPLC method are available for the drug.

The Triple Pak combination of the drugs Amoxicillin, Clarithromycin, Vonoprazan, is used in the the therapy of the *Helicobacter pylori* infections employs the use of new combinational these drugs. The Triple Pack drug combination is effective in the treatment as the drugs Amoxicillin, Clarithromycin are anti-microbial agents eradicates the microbial infections. Vonoprazan drug is competitive acid blocker helps in the treating hyper acidity and gastric-duodenal ulcer’s along with the combination with Amoxicillin and Clarithromycin induced by *Helicobacter pylori* infections. The review of literature for these drugs shows, HPLC, LC/MS, UV spectroscopic methods for Amoxicillin, Clarithromycin with other combinations like ornidazole, metronidazole, tinidazole, omeprazole, and other drugs. There are no reported methods for with vonoprazan drug in the triple pak combination.

Chapter 4 – HPLC Method Development And Validation For Delstrigo Combination

The The Analytical HPLC method for LAM, TEN & DOR combinational drugs has been successfully developed and validated. The analytical method is optimized in testing,analysis of these selected drugs in individual as well in the combined for ms and all validation parameters., are performed in the acceptance criteria as per ICH regulatory guideline. The summarized results are shown below:

Parameters	Conditions
Stationary Phase Coloumn	C18 Hypersil BDS 250 x 4.6mm , 5 micron
Mobile phase	Phosphate buffer : ACN 80:20 pH-3.5
Flow rate	1ml/min
Injection volume	20ul
Temp	Ambient Lab Temperature
Detection Wavelength	269nm
Retention Times (min)	LAM-5.77, TEN-6.78, DOR-8.39

Sr No	Parameters	Results		
		LAM	TEN	DOR
1	System Suitability: Theoretical plates- Tailing Factor- Retention time min-	 4651 1.07 5.77	 6394 1.23 6.78	 7203 1.16 8.39
2	Precision (%RSD)	0.31	0.32	0.49
3	Linearity (R^2)	0.9994	0.9994	0.9991
4	Accuracy (% Recovery)	99.33	99.68	98.95
5	LOD (ug/ml)	1.23	1.26	0.52
6	LOQ (ug/ml)	3.75	3.82	1.59
7	% Assay	92.41	97.66	97.82
8	Dissolution % Drug Release at 40min	99.26	98.97	98.53

Chapter 5 – Stability Indicating HPLC Method Development And Validation For Delstrigo Combination

The stability Analytical HPLC method for LAM, TEN & DOR combinational drugs has been successfully developed and validated. The analytical method is optimized in testing,analysis of these selected drugs in individual as w ell in the combined forms and all validation parameters., are performed in the acceptance criteria as per ICH regulatory guideline. The summarized results are shown below:

Parameters	Conditions
Stationary Phase Coloumn	C18 Hypersil BDS 250 x 4.6mm , 5 micron
Mobile phase	Phosphate buffer : ACN 80:20 pH-3.5
Flow rate	1ml/min
Injection volume	20ul
Temp	Ambient Lab Temperature
Detection Wavelength	269nm
Retention Times (min)	LAM-5.76, TEN-6.79, DOR-8.37

Sr No	Parameters	Results		
		LAM	TEN	DOR
1	System Suitability: Theoretical plates- Tailing Factor- Retention time min-	 4532 1.09 5.76	 6283 1.21 6.79	 7193 1.19 8.38
2	Precision (%RSD)	0.54	0.32	0.64
3	Linearity (R^2)	0.9996	0.9995	0.9998
4	Accuracy (% Recovery)	99.43	99.38	100.24
5	LOD (ug/ml)	1.49	1.60	0.57
6	LOQ (ug/ml)	4.53	4.87	1.73
7	% Assay	96.25	96.03	97.47
8	Dissolution % Drug Release at 40min	99.26	98.97	98.53

Chapter 6 –HPLC Method Development And Validation For Cabotegravir And Rilpivirine

The Analytical HPLC method for CAB & RILP combinational drugs has been successfully developed and validated. The analytical method is optimized in testing, analysis of these selected drugs in individual as well in the combined forms and all validation parameters., are performed in the acceptance criteria as per ICH guideline. The summarized results are shown below:

Parameters	Conditions
Stationary Phase Coloumn	C18 Hypersil BDS 250 x 4.6mm , 5 micron
Mobile phase	Phosphate buffer : ACN 65:35 pH-5.5
Flow rate	1ml/min
Injection volume	20ul
Temp	Ambient Lab Temperature
Detection Wavelength	242.5nm
Retention Times (min)	CAB-2.14, RILP-3.21

Sr No	Parameters	Results	
		CAB	RILP
1	System Suitability: Theoretical plates- Tailing Factor- Retention time min-	 5412 1.31 2.14	 7644 1.24 3.21
2	Precision (%RSD)	0.12	0.10
3	Linearity (R^2)	0.9999	0.9999
4	Accuracy (% Recovery)	99.44	100.23
5	LOD (ug/ml)	0.26	0.20
6	LOQ (ug/ml)	0.79	0.61
7	% Assay	99.80	99.95

Chapter 7 –Stability Indicating HPLC Method Development And Validation For Cabotegravir And Rilpivirine

The Stability Analytical HPLC method for CAB & RILP combinational drugs has been successfully developed and validated. The analytical method is optimized in testing,analysis of these selected drugs in individual as w ell in the combined forms and all validation parameters., are performed in the acceptance criteria as per ICH guideline. The summarized results are shown below:

Parameters	Conditions
Stationary Phase Coloumn	C18 Hypersil BDS 250 x 4.6mm , 5 micron
Mobile phase	Phosphate buffer : ACN 65:35 pH-5.5
Flow rate	1ml/min
Injection volume	20ul
Temp	Ambient Lab Temperature
Detection Wavelength	242.5nm
Retention Times (min)	CAB-2.142, RILP-3.214

Sr No	Parameters	Results	
		CAB	RILP
1	System Suitability: Theoretical plates- Tailing Factor- Retention time min-	 5421 1.31 2.14	 7464 1.24 3.21
2	Precision (%RSD)	0.12	0.10
3	Linearity (R^2)	0.9999	0.9999
4	Accuracy (% Recovery)	99.78	100.25
5	LOD (ug/ml)	0.23	0.21
6	LOQ (ug/ml)	0.71	0.64
7	% Assay	99.0	99.13

Chapter 8 – HPLC Method Development And Validation For Dolutegravir Tenofovir And Rilpivirine

The Analytical HPLC method for DOLU, TEN & RILP combinational drugs has been successfully developed and validated. The analytical method is optimized in testing,analysis of these selected drugs in individual as w ell in the combined forms and all validation parameters., are performed in the acceptance criteria as per ICH regulatory guideline. The summarized results are shown below:

Parameters	Conditions
Stationary Phase Coloumn	C18 Hypersil BDS 250 x 4.6mm , 5 micron
Mobile phase	Phosphate buffer pH- 3.5 : ACN : Methanol 65:15:20
Flow rate	1ml/min
Injection volume	20ul
Temp	Ambient Lab Temperature
Detection Wavelength	229.6nm
Retention Times (min)	DOLU-2.53, TEN-3.46, RILP-4.76

Sr No	Parameters	Results		
		DOLU	TEN	RILP
1	System Suitability: Theoretical plates- Tailing Factor- Retention time min-	 5537 1.41 2.53	 5537 1.41 2.53	 5537 1.41 2.53
2	Precision (%RSD)	0.50	0.50	0.50
3	Linearity (R^2)	0.9999	0.9999	0.9999
4	Accuracy (% Recovery)	98.98	98.98	98.98
5	LOD (ug/ml)	0.24	0.24	0.24
6	LOQ (ug/ml)	0.75	0.75	0.75
7	% Assay	98.01	98.01	98.01

Chapter 9 – Stability HPLC Method Development And Validation For Dolutegravir Tenofovir And Rilpivirine

The Stability Analytical HPLC method for DOLU, TEN & RILP combinational drugs has been successfully developed and validated. The analytical method is optimized in testing,analysis of these selected drugs in individual as w ell in the combined forms and all validation parameters., are performed in the acceptance criteria as per ICH regulatory guideline. The summarized results are shown below:

Parameters	Conditions
Stationary Phase Coloumn	C18 Hypersil BDS 250 x 4.6mm , 5 micron
Mobile phase	Phosphate buffer pH- 3.5 : ACN : Methanol 65:15:20
Flow rate	1ml/min
Injection volume	20ul
Temp	Ambient Lab Temperature
Detection Wavelength	229.6nm
Retention Times (min)	DOLU-2.53, TEN-3.46, RILP-4.76

Sr No	Parameters	Results		
		DOLU	TEN	RILP
1	System Suitability: Theoretical plates- Tailing Factor- Retention time min-	 5537 1.41 2.53	 6772 1.21 3.46	 7781 1.32 4.76
2	Precision (%RSD)	0.42	0.23	0.14
3	Linearity (R^2)	0.9999	0.9999	0.9998
4	Accuracy (% Recovery)	99.78	99.29	100.28
5	LOD (ug/ml)	0.31	1.29	0.13
6	LOQ (ug/ml)	0.96	3.92	0.40
7	% Assay	98.03	99.01	98.25

Chapter 10 – HPLC Method Development And Validation For Fexinidazole

The Analytical HPLC method for FEXI drugs has been successfully developed and validated. The analytical method is optimized in testing,analysis of these selected drugs in individual as well in the combined forms and all validation parameters., are performed in the acceptance criteria as per ICH regulatory guideline. The summarized results are shown below:

Parameters	Conditions
Stationary Phase Coloumn	C18 Hypersil BDS 250 x 4.6mm , 5 micron
Mobile phase	Phosphate buffer pH- 4.5 : ACN 35:65
Flow rate	1ml/min
Injection volume	20ul
Temp	Ambient Lab Temperature
Detection Wavelength	255.5nm
Retention Times (min)	FEXI-2.45

Sr No	Parameters	Results FEXI
1	System Suitability: Theoretical plates- Tailing Factor- Retention time min-	 3872 1.34 2.45
2	Precision (%RSD)	0.54
3	Linearity (R^2)	0.9999
4	Accuracy (% Recovery)	99.64
5	LOD (ug/ml)	0.46
6	LOQ (ug/ml)	1.39
7	% Assay	97.91
8	Dissolution % Drug Release at 40min	97.92

Chapter 11 – Stability Indicating HPLC Method Development And Validation For Fexinidazole

The Stability Analytical HPLC method for FEXI drugs has been successfully developed and validated. The analytical method is optimized in testing,analysis of these selected drugs in individual as well in the combined forms and all validation parameters., are performed in the acceptance criteria as per ICH regulatory guideline. The summarized results are shown below:

Parameters	Conditions
Stationary Phase Coloumn	C18 Hypersil BDS 250 x 4.6mm , 5 micron
Mobile phase	Phosphate buffer pH- 4.5 : ACN 35:65
Flow rate	1ml/min
Injection volume	20ul
Temp	Ambient Lab Temperature
Detection Wavelength	255.5nm
Retention Times (min)	FEXI-2.451

Sr No	Parameters	Results FEXI
1	System Suitability: Theoretical plates- Tailing Factor- Retention time min-	 3827 1.29 2.45
2	Precision (%RSD)	0.64
3	Linearity (R^2)	0.9999
4	Accuracy (% Recovery)	100.42
5	LOD (ug/ml)	0.59
6	LOQ (ug/ml)	1.79
7	% Assay	97.70
8	Dissolution % Drug Release at 40min	97.91

Chapter 12 –HPLC Method Development And Validation For Maribavir

The Analytical HPLC method for MARI drugs has been successfully developed and validated. The analytical method is optimized in testing,analysis of these selected drugs in individual as well in the combined forms and all validation parameters., are performed in the acceptance criteria as per ICH regulatory guideline. The summarized results are shown below:

Parameters	Conditions
Stationary Phase Coloumn	C18 Hypersil BDS 250 x 4.6mm , 5 micron
Mobile phase	ACN : Phosphate buffer pH- 4 (15:85)
Flow rate	1ml/min
Injection volume	20ul
Temp	Ambient Lab Temperature
Detection Wavelength	243nm
Retention Times (min)	MARI-3.13

Sr No	Parameters	Results MARI
1	System Suitability: Theoretical plates- Tailing Factor- Retention time min-	 3641 1.27 3.13
2	Precision (%RSD)	0.48
3	Linearity (R^2)	0.999
4	Accuracy (% Recovery)	99.79
5	LOD (ug/ml)	0.306
6	LOQ (ug/ml)	0.927
7	% Assay	97.73

Chapter 13 –Stability Indicating HPLC Method Development And Validation For Maribavir

The Stability Analytical HPLC method for MARI drugs has been successfully developed and validated. The analytical method is optimized in testing,analysis of these selected drugs in individual as well in the combined forms and all validation parameters., are performed in the acceptance criteria as per ICH regulatory guideline. The summarized results are shown below:

Parameters	Conditions
Stationary Phase Coloumn	C18 Hypersil BDS 250 x 4.6mm , 5 micron
Mobile phase	ACN : Phosphate buffer pH- 4 (15:85)
Flow rate	1ml/min
Injection volume	20ul
Temp	Ambient Lab Temperature
Detection Wavelength	243nm
Retention Times (min)	MARI-3.132

Sr No	Parameters	Results MARI
1	System Suitability: Theoretical plates- Tailing Factor- Retention time min-	 3614 1.26 3.13
2	Precision (%RSD)	0.21
3	Linearity (R^2)	0.999
4	Accuracy (% Recovery)	99.20
5	LOD (ug/ml)	0.307
6	LOQ (ug/ml)	0.930
7	% Assay	98.37

Chapter 14 –HPLC Method Development And Validation For Molnupiravir

The Analytical HPLC method for MOLN drugs has been successfully developed and validated. The analytical method is optimized in testing,analysis of these selected drugs in individual as well in the combined forms and all validation parameters., are performed in the acceptance criteria as per ICH regulatory guideline. The summarized results are shown below:

Parameters	Conditions
Stationary Phase Coloumn	C18 Hypersil BDS 250 x 4.6mm , 5 micron
Mobile phase	ACN : Phosphate buffer pH- 3 (35:65)
Flow rate	1ml/min
Injection volume	20ul
Temp	Ambient Lab Temperature
Detection Wavelength	236nm
Retention Times (min)	MOLN-2.53

Sr No	Parameters	Results MOLN
1	System Suitability: Theoretical plates- Tailing Factor- Retention time min-	 3985 1.21 2.53
2	Precision (%RSD)	0.21
3	Linearity (R^2)	0.999
4	Accuracy (% Recovery)	99.45
5	LOD (ug/ml)	1.181
6	LOQ (ug/ml)	3.581
7	% Assay	98.20

Chapter 15 –Stability Indicating HPLC Method Development And Validation For Molnupiravir

The Stability Indicating Analytical HPLC method for MOLN drugs has been successfully developed and validated. The analytical method is optimized in testin g,analysis of these selected drugs in individual as well in the combined forms and all validation parameters., are performed in the acceptance criteria as per ICH regulatory guideline. The summarized results are shown below:

Parameters	Conditions
Stationary Phase Coloumn	C18 Hypersil BDS 250 x 4.6mm , 5 micron
Mobile phase	ACN : Phosphate buffer pH- 3 (35:65)
Flow rate	1ml/min
Injection volume	20ul
Temp	Ambient Lab Temperature
Detection Wavelength	236nm
Retention Times (min)	MOLN-2.532

Sr No	Parameters	Results MOLN
1	System Suitability: Theoretical plates- Tailing Factor- Retention time min-	 3958 1.203 2.532
2	Precision (%RSD)	0.61
3	Linearity (R^2)	0.999
4	Accuracy (% Recovery)	100.35
5	LOD (ug/ml)	0.92
6	LOQ (ug/ml)	2.81
7	% Assay	98.27

Chapter 16 –HPLC Method Development And Validation For Voquezna Combination Amoxicillin Clarithromycin And Vonoprazan

The Analytical HPLC method for AMOX, CLAR & VONO combinational drugs has been successfully developed and validated. The analytical method is optimized in testing,analysis of these selected drugs in individual as w ell in the combined forms and all validation parameters., are performed in the acceptance criteria as per ICH regulatory guideline. The summarized results are shown below:

Parameters	Conditions
Stationary Phase Coloumn	C18 Hypersil BDS 250 x 4.6mm , 5 micron
Mobile phase	Phosphate buffer pH- 3.5 : ACN : Methanol 80:15:5 with 0.1% TEA
Flow rate	1ml/min
Injection volume	20ul
Temp	Ambient Lab Temperature
Detection Wavelength	229nm
Retention Times (min)	AMOX-2.75, CLAR-4.23, VONO-5.57

Sr No	Parameters	Results		
		AMOX	CLAR	VONO
1	System Suitability: Theoretical plates- Tailing Factor- Retention time min-	 4063 1.12 2.75	 6417 1.37 4.23	 7358 1.29 5.57
2	Precision (%RSD)	0.24	0.40	0.39
3	Linearity (R^2)	0.999	0.999	0.999
4	Accuracy (% Recovery)	100.20	98.74	99.58
5	LOD (ug/ml)	1.124	1.453	0.248
6	LOQ (ug/ml)	3.408	4.403	0.753
7	% Assay	98.14	98.09	99.87

Chapter 17 –Satbility HPLC Method Development And Validation For Voquezna Combination Amoxicillin Clarithromycin And Vonoprazan

The Stability Analytical HPLC method for AMOX, CLAR & VONO combinational drugs has been successfully developed and validated. The analytical method is optimized in testing,analysis of these selected drugs in individual as w ell in the combined forms and all validation parameters., are performed in the acceptance criteria as per ICH regulatory guideline. The summarized results are shown below:

Parameters	Conditions
Stationary Phase Coloumn	C18 Hypersil BDS 250 x 4.6mm , 5 micron
Mobile phase	Phosphate buffer pH- 3.5 : ACN : Methanol 80:15:5 with 0.1% TEA
Flow rate	1ml/min
Injection volume	20ul
Temp	Ambient Lab Temperature
Detection Wavelength	229nm
Retention Times (min)	AMOX-2.752, CLAR-4.233, VONO-5.572

Sr No	Parameters	Results		
		AMOX	CLAR	VONO
1	System Suitability: Theoretical plates- Tailing Factor- Retention time min-	 4136 1.123 2.751	 6371 1.314 4.230	 7289 1.227 5.573
2	Precision (%RSD)	0.27	0.56	0.32
3	Linearity (R^2)	0.9998	0.9996	0.9999
4	Accuracy (% Recovery)	101.49	99.03	100.40
5	LOD (ug/ml)	1.46	1.41	0.244
6	LOQ (ug/ml)	4.44	4.28	0.740
7	% Assay	99.08	97.93	99.94

Conclusion

The developed analytical methods are able to detect the drugs and their combinations in individual as well as in the different dosage forms with the accuracy and precision parameters as per the ICH guideline. The Stability method i.e. forced degradation study helps to understand the different impurities, degraded products, generated in stress conditions and the method effectively detects the drugs analytes pure peaks, without any interference of other peaks. The method is effectively validated as per the ICH guidelines, so is useful for the assay and recovery study from the marketed formulations as well as effectively used in vitro dissolution profile study in oral dosage forms. The HLPC run time is shorter which is beneficial and time saving for the quick analysis of the drugs in different individual dosage-forms as well as in the combinations.

The main research objectives of Analytical Method development & Validation by adopting spectroscopic & chromatographic techniques for the selected class of Antimicrobial Agents & their combinations in pure api synthetic mixture and assay method for the pharmaceutical dosage forms have been successfully achieved and the research problem domain has been effectively solved by carrying out the research work. The research work bridges the gap and solves the problems that are present in the current pharmaceuticals, industrial, academic fields.