SYNOPSIS OF Ph.D. THESIS ENTITLED

“DEVELOPMENT AND VALIDATION OF NEW ANALYTICAL METHOD FOR BIOACTIVE COMPOUNDS”

SUBMITTED TO
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GANDHINAGAR, GUJARAT, INDIA

FOR THE DEGREE OF
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IN
CHEMISTRY

BY
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UNDER THE SUPERVISION OF
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FACULTY : CHEMISTRY

SUBJECT : CHEMISTRY

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A comprehensive summary of the research work to be incorporated in the thesis entitled “DEVELOPMENT AND VALIDATION OF NEW ANALYTICAL METHOD FOR BIOACTIVE COMPOUNDS” has been described as under.

**Chapter: 1. Introduction**

High Performance Liquid Chromatography (HPLC)
Ultra Performance Liquid Chromatography (UPLC)

**Chapter: 2. Analytical/UPLC Method Development and Validation**

Analytical Method Development
Analytical Method Validation

**Chapter: 3. Simultaneous Determination of Ambroxol Hydrochloride, Cetirizine Hydrochloride, Methylparaben and Propylparaben in Liquid Pharmaceutical Formulation.**

The research work undertaken in this study, in the area of pharmaceuticals and mainly addresses method development for simultaneous determination of Ambroxol Hydrochloride (AMB), Cetirizine Hydrochloride (CTZ), Methylparaben (MP) and Propylparaben (PP) in drug product by UPLC. Developed analytical method was validated for its intended application, as per ICH guidelines.

**Aim of work:**


**Chemical structure, UV spectra and IUPAC name of AMB, CTZ, MP and PP**

Ambroxol hydrochloride (AMB)

\[
\text{trans-4-[(2-amino-3,5-dibromobenzyl)amino]cyclohexanol hydrochloride}
\]

![Chemical structure of Ambroxol hydrochloride](image1)

![UV Spectrum of Ambroxol hydrochloride](image2)
Optimised chromatographic conditions are as under:

Mobile phase-A: Mixture of 0.01M phosphate buffer (KH$_2$PO$_4$) in 0.1% triethylamine
Mobile phase-B: Acetonitrile
Mobile phase elution: Gradient
Column: Agilent Eclipse Plus C18, RRHD 1.8 μm (50 mm x 2.1 mm)
Flow rate: 0.5 mL/min
Wavelength: 237 nm
Injection volume: 4 μL
Column oven temp: 50 °C
Sample temp: 25°C
Diluent: Mixture of water and methanol in the ratio of 50:50 (v/v)
Developed method specimen chromatograms are presented in Figure 1 and 2.

**Fig. 1.** Overlay chromatograms of placebo, blank and spiked impurities along with analytes

**Fig. 2.** Overlay chromatograms of blank, placebo and sample preparation

A gradient RP-UPLC method was successfully developed for the simultaneous estimation of AMB, CTZ, MP and PP in liquid pharmaceutical formulation. The developed method is selective, precise, accurate, linear, filter compatible and robust. Forced degradation data proved that the method is specific for the analytes and free from the interference of placebo/known impurities/degradation products and unknown degradation products. The run time (3.5 min) enables rapid determination of drugs and preservatives. Moreover, it may be applied for individual and simultaneous determination of AMB, CTZ, *levo*-Cetirizine (LCTZ), MP and PP compound in pharmaceutical drug product and substance. Also it can be utilized for determination of assay, blend uniformity and content uniformity of pharmaceutical products (CTZ tablets and AMB+CTZ tablets), where sample load is higher and high throughput is essential for faster delivery of results.
Chapter: 4. Determination of Quetiapine in Pharmaceutical Dosage Form

The research work undertaken in this study, in the area of pharmaceuticals and mainly addresses method development for determination of Quetiapine (QUE) by UPLC. Developed analytical method was validated for its intended application, as per ICH guidelines.

Aim of work:
Development and validation of a stability indicating reversed phase ultra performance liquid chromatography (RP-UPLC) method for determination of QUE in solid oral dosage form.

Chemical structure, UV spectra and IUPAC name of Quetiapine and Impurities

1. Quetiapine (QUE)

2. Quetiapine-N-Oxide (N-Oxide)

3. Quetiapine-S-Oxide (S-Oxide)

4. Quetiapine-IV (Que-IV)
Optimised chromatographic conditions are as under:

Mobile phase-A : 0.1% aqueous triethylamine (adjusted pH 7.2, with orthophosphoric acid)
Mobile phase-B : Mixture of acetonitrile and methanol in the ratio of 80:20 (v/v)
Mobile phase elution : Gradient
Column : Agilent Eclipse Plus C18, RRHD 1.8 μm (50 mm x 2.1 mm)
Flow rate : 0.5 mL/min
Wavelength : 252 nm
Injection volume : 1 μL
Column oven temp : 40 °C
Sample temp : 25°C
Diluent : Water, Acetonitrile and Perchloric acid in the ratio of 200:800:0.13 (v/v/v)

Developed method specimen chromatograms are presented in Figure 3 and 4.
A gradient RP-UPLC method was successfully developed for the estimation of quetiapine in pharmaceutical dosage form. The method validation results have proved that the method is selective, precise, accurate, linear, robust, filter compatible and stability indicating. The run time (5.0 min) enables rapid determination of drug. Moreover, it may be applied for determination of QUE in the study of blend uniformity, tablet content uniformity and in-vitro dissolution profiling of QUE dosage forms, where sample load is higher and high throughput is essential for faster delivery of results.

Chapter: 5. Aniline/Genotoxic Impurity Determination in Mesalamine Delayed Release Tablets by UPLC

The research work undertaken in this study, in the area of pharmaceuticals and mainly addresses method development for determination of Aniline/Genotoxic impurity in Mesalamine delayed release tablets by UPLC. Developed analytical method was validated for its intended application, as per ICH guidelines.

Aim of work:


Chemical structure, UV spectra and IUPAC name of Aniline and Mesalamine
SYNOPSIS

Aniline; Phenylamine; Aminobenzene, Benzenamine

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{Aniline} \\
& \\
\text{HO} & \quad \text{Mesalamine; Mesalazine; 5-Aminosalisilic acid; 5-ASA} \\
\text{HO} & \\
\text{O} & \quad \text{5-Amino-2-hydroxybenzoic acid}
\end{align*}
\]

Optimised chromatographic conditions are as under:

Buffer : 1.74 g of (K\textsubscript{2}HPO\textsubscript{4}) and 2.2 g of 1-octane sulphonic acid sodium salt was dissolved in one litre of milli-Q water. Adjusted pH 6.0 with orthophosphoric acid. 
Mobile phase : Mixture of buffer and acetonitrile in the ratio of 90:10 (v/v)
Mobile phase elution : Isocratic
Column : Reprosil Gold 100, C18-XBD, 50 mm x 2.0 mm, 1.8 μm
Flow rate : 0.5 mL/min
Wavelength : 200 nm
Injection volume : 7 μL
Column oven temp : 50 °C
Sample temp : 25°C
Diluent : 1N hydrochloric acid and 1N sodium hydroxide solution in the ratio of 50:50 (v/v)

Developed method specimen chromatogram is presented in Figure 5.

![Fig. 5. Overlay chromatogram of blank (bottom), placebo (middle) and standard preparation (top)](image-url)
A rapid, RP-UPLC method was successfully developed and validated for determination of Aniline impurity from mesalamine drug product. The total run time was 5min, within which drug and their degradation products were separated from aniline. Method validation results have proved that the method is selective, precise, accurate, linear, rugged, robust and stability indicating with low LOD and LOQ. Sample solution stability and filter compatibility was also established. This method can successfully be applied for routine analysis and stability study of mesalamine delayed release drug product. Thus, the method provides high throughput solution for determination of aniline in the mesalamine delayed release formulation with excellent selectivity, precision and accuracy. This method can also be applied to quantify the trace levels of aniline from drug substances, drug products and different type of samples.

Chapter: 6. Determination of Mesalamine Related Impurities in Mesalamine Delayed Release Tablets

The research work undertaken in this study, in the area of pharmaceuticals and mainly addresses method development for determination of Mesalamine related impurities by UPLC. Developed analytical method was validated for its intended application, as per ICH guidelines.

**Aim of work:**

Development and validation of a stability indicating reversed phase ultra performance liquid chromatography (RP-UPLC) method for determination of impurities in Mesalamine solid oral dosage form.

**Chemical structure and IUPAC name of Mesalamine and its impurities**

*Mesalamine*  
5-amino-2-hydroxybenzoeic acid  
*Impurity-C*  
2,5-dihydroxy benzoeic acid  
*Impurity-D*  
2-hydroxy benzoeic acid  
*Impurity-E*  
3-Amino salicylic acid  
*Impurity-F*  
3-Amino benzoeic acid  
*Impurity-G*
Optimised chromatographic conditions are as under:

Mobile phase-A : Buffer pH 2.2
Mobile phase-B : Buffer (pH 6.0), methanol and acetonitrile in the ratio of 890:80:30 (v/v/v)
Mobile phase elution : Gradient
Column : Acquity UPLC BEH C18 (50 mm x 2.1 mm, 1.7 µm)
Flow rate : 0.7 mL/min
Wavelength : 220 nm
Injection volume : 7 µL
Column oven temp : 40 °C
Sample temp : 25°C
Diluent-1 : 1N hydrochloric acid
Diluent-2 : Buffer (pH 2.2), methanol and acetonitrile in the ratio of 890:80:30 (v/v/v)

Developed method specimen chromatograms are presented in Figure 6 and 7.

Fig. 6. Overlay chromatograms of placebo (bottom) and spiked impurities (top) with its 3D plot
A new RP-UPLC method was successfully developed and validated for simultaneous
determination of all six impurities from mesalamine delayed-release formulation. The total
run time was 15 minutes, within which drug and their impurities/degradation products were
well separated with each other. Method validation results have proved that the method is
selective, precise, accurate, linear, rugged, robust and stability indicating with low LOD and
LOQ. This method can be successfully applied for the routine analysis as well as stability
study of mesalamine delayed-release drug product. Overall, the method provides high
throughput solution for determination of all related impurities in mesalamine delayed-
release formulation with excellent selectivity, precision and accuracy.

Chapter: 7. Determination of Metaxalone and its Impurities in Solid Oral Dosage Form

The research work undertaken in this study, in the area of pharmaceuticals and mainly
addresses method development for determination of Metaxalone and its impurities by
UPLC. Developed analytical method was validated for its intended application, as per ICH
guidelines.

Aim of work:

Development and validation of a stability indicating reversed phase ultra performance
liquid chromatography (RP-UPLC) method for determination of Metaxalone and its
impurities in Metaxalone solid oral dosage form.

Chemical structures, UV spectrums and IUPAC name of META, Imp-A and Imp-B
Optimised chromatographic conditions parameters are as under:

Mobile phase: Water, acetonitrile, methanol and triethylamine in the ratio of 50:25:25:0.1 (v/v/v/v), pH was adjusted 6.3 with orthophosphoric acid

Mobile phase elution: Isocratic
Column: Acquity® HSS-T3 (100 mm x 2.1 mm, 1.8 µm)
Flow rate: 0.3 mL/min
Wavelength: 230 nm (for Assay) and 205 (for RS)
Injection volume: 1 µL
Column oven temp: 45 °C
Sample temp: 25°C
Diluent: Mobile phase

Developed method specimen chromatograms are presented in Figure 8 and 9.
The rapid isocratic RP-UPLC method was developed for quantitative and related substances analysis of Metaxalone in pharmaceutical formulation. Satisfactory results were obtained from validation of the method. The run time (6 min) enabled rapid determination of META. This method exhibited an excellent performance in terms of sensitivity and speed. This stability-indicating method can be applied for the routine analysis of production samples and to check the stability of Metaxalone in bulk drug and formulation. Moreover, it can be applied for determination of assay, blend uniformity, content uniformity and in vitro dissolutions of pharmaceutical products, where sample load is higher and high throughput is essential for faster delivery of results.
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