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R E S E A R C H A R T I C L E

Analytical Method development for the Estimation of Related Substances by High performance Liquid Chromatographyfor the Rimonabent (RIM) drug

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A B S T R A C T

The main aim of present research work analytical method development for the estimation of related substances by high performance liquid chromatography for the Rimonabent drug.'RIM' is anti-obesity drug, a proper analytical method is required to separate and quantify it's all related substances or impurities. The substances are separated by four stages. Each and every stage various trials were conducted by HPLC by using different chromatographic conditions. The developed new or improved method usually tailors existing approaches and instrumentation to the current analyte, as well as to the final needs or requirements and deciding on instrumentation to utilize in the development stage decisions regarding choice of column, mobile phase, detectors and method of quantification must be addressed.

Key words: Rimonabent (RIM), HPLC, Mobile phase, Column, analyte, related substances.

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Impurities:

ICH has given guide lines by its expert working group. these guide lines are intended to provide guidance to identify the content and qualification of impurities in drug substances.Impurities in new drug substance are addressed from two perspectives: Chemistry aspects and safety

aspects. Impurities can be classified into following categories;

- Organic impurities
- Inorganic impurities
- Residual impurities

Organic impurities:

The actual and potential impurities most likely to arise during the synthesis, purification and storage of new drug substance should be summarized .This summary should be based on sound scientific appraisal of the chemical reactions involved in synthesis, impurities associated with raw materials that could contribute to the impurity profile of the new drug substance,and possible degradation products.

Inorganic impurities:

Inorganic impurities are normally detected and quantified using Pharmacopeial or other appropriate procedures.Carryover of catalysts to the new drug substance should be evaluated during development. The need for inclusion or exclusion of inorganic impurities in the drug substance specification should be discussed.Acceptance criteria should be based on Pharmacopeia standards or known safety data.

Residual Impurities (Solvents):

The control of residues of the solvents used in the manufacture process for the drug substance should be given according to the ICH guidelines.

Analytical Methods for Impurities:

To quantify and identify the impurities in pharmaceutical products there are various analytical methods like spectrophotometric methods, flourmetric methods and chromatographic techniques out of these methods chromatographic techniques are more efficient and sensitive, these techniques are widely used for method development for quantification and identification of impurities.

High Performance Liquid Chromatography:

HPLC is defined as High Performance Liquid Chromatography or High Pressure Liquid Chromatography. In HPLC separations are achieved by partition, adsorption or ion exchange depending on the stationary phase.HPLC is advantageous over Gas chromatography in analysis of organic compounds because the compounds are dissolved in organic liquid and most of the separations take place at room temperature. Non-volatile and thermally unstable drugs can be chromatographed without decomposition or necessity of making volatile derivatives.Systems with polar stationary phases and non-polar mobile phases are called normal phases and those with non-polar stationary phases and polar mobile phases are known as reverse phases.

Drug profile:

Rimonabant is a specific CB1 cannabinoid receptor antagonist. There is considerable evidence that the endocannabinoid (endogenous cannabinoid) system plays a significant role in appetitive drive and associated behaviours. It is therefore reasonable to hypothesize that the attenuation of the activity of this system would have therapeutic benefit in treating disorders that might have a component of excess appetitive drive or over-activity of the endocannabinoid system, such as obesity, ethanol and other drug abuse, and a variety of central nervous system and other disorders.

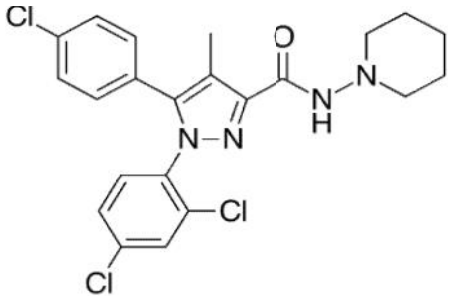

Fig 1: Structure of Rimonabant

2. Materials and Methods

Table 1: List of Chemicals

Reagents	Company
Acetonitrile - HPLC Grade	Rankem
Potassium di hydrogen phosphate-HPLC Grade	Merck
Ethanol	Merck
Ortho Phosphoric acid	Fluka
Triethylamine	Tedia

Instruments Used:

HPLC –WATERS **:** With Waters 2996 PDA Detector (Empower-Software)
HPLC-AGILENT **:** With Waters 2996 PDA Detector (Empower-Software)
Balances **:** Sartorius
Degasser **:** Milli Q
Sonicator **:** Bandelin Sonicator
pH Meter **:** Eutech pH Meter
HPLC Columns**:** Hypersil BDS C-18, 250x4.6; 5µ
Inertsil ODS 250x4.6x5µ,
Hypersil Gold, 150x4.6; 3µ.

3. Results and discussion

Various trials were conducted for the separation of Rimonabant drug related substances and impurities in different chromatographic conditions by using HPLC.

Experiment Trail No: 1

Chromatographic Conditions:

Column **:**Xterra. RP 18 250*4.6 5 micron
Flow **:**1.0 ml/min
Column Oven Temperature **:** Ambient
Wave length **:**254 nm
Injection Volume **:** 10 µl
Run time **:**60 min
Diluent **:**Ethanol
Sample Preparation **:**2 mg/ml

Mobile Phase Preparation:

Mobile Phase A:(20: 80:1ml) Water: Acetonitrile: TEA
Mobile Phase B:(50: 50: 1ml) Water: Acetonitrile: TEA
Mix well, filtered and degassed the Mobile Phase.

Gradient Programme:

Time in min	% of A	% of B
0.01	100	0
8.0	100	0
20.00	0	100
50.00	0	100
52.00	100	0
60.00	100	0

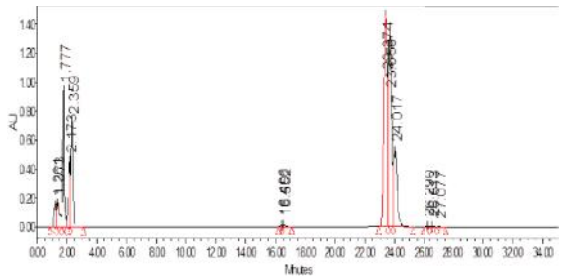

Fig 2:RIM-1D Chromatogram

Observation:
Starting material peak is splitting and merging with two other peaks. And Peak shapes were not good.

Experiment Trail No: 2
Chromatographic Conditions:
Column**:** Inertsil ODS, 3V 250 x 4.6 x 5µ.
Flow**:** 1 ml/min
Column Oven
Temperature**:** Ambient
Wave length **:** 254 nm
Injection Volume **:** 10 µl
Run time **:** 60 min
Diluent **:** Ethanol
Sample Preparation **:** 2.0 mg/ml
Mobile Phase Preparation:
Buffer: 0.05M (7.80 g) of NaH_2PO_4 is dissolved in 1000 of Milli Q water, and pH Adjusted to 2.5 with H3PO4 .
Mobile Phase A : (80: 20) Buffer: Acetonitrile
Mobile Phase B : (20: 80) Water: Acetonitrile
Mix well, filtered and degassed the Mobile Phase.
Gradient Programme:

Time in min	% of A	% of B
0.00	70	30
5.00	70	30
35.00	15	85
50.00	15	85
52.00	70	30
60.00	70	30

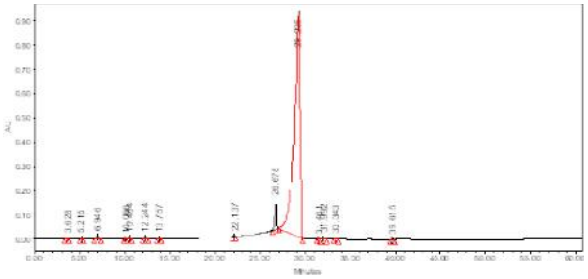

Fig 3:RIM-1D & HOBT Chromatogram

Observation:
In these conditions starting material and Rim-1D are separating well. Rim-1D peak shape is not good.

Experiment Trail No: 3
Chromatographic Conditions:
Column **:** Inertsil ODS, 3V 150 x 4.6 5µ.
Flow **:**1.2 ml/min
Column Oven
Temperature**:**Ambient
Wave length **:**215 nm
Injection Volume **:** 10 µl
Run time **:**60 min
Diluent **:**Mobile phase-B
Sample Preparation **:**0.5 mg/ml

Mobile Phase Preparation:
Buffer: 0.02M (2.72 g) of KH2PO4 is dissolved in 1000ml of milli Q water, and adjust the pH 4.0 with H_3PO_4.
Mobile Phase A : (80: 20) Buffer: Acetonitrile
Mobile Phase B : (20: 80) Buffer: Acetonitrile
Mix well, filtered and degassed the Mobile Phase.
Gradient Programme:

Time in min	% of A	% of B
0.00	70	30
5.00	70	30
35.00	15	85
50.00	15	85
52.00	70	30
60.00	70	30

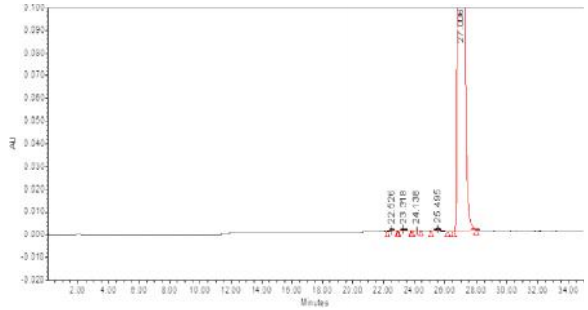

Fig 4: RIM-3D Chromatogram

Observation:
In the above chromatogram unknown Impurity before main peak is separating well and Hydrazine impurity is not eluting. Ester impurity is closely eluted.

Experiment Trail No: 4
Chromatographic Conditions:
Column **:** Inertsil ODS, 150 x 4.6 x 5µ.
Flow **:** 1.2 ml/min
Column Oven
Temperature **:** Ambient
Wave length **:** 215 nm
Injection Volume **:** 10 µl
Run time**:** 60 min
Diluent **:** MP-B
Sample Preparation **:** 0.5 mg/ml

Mobile Phase Preparation:

Buffer: 0.02M (2.72 g) of KH_2PO_4 is dissolved in 1000ml of milli Q water, and adjust the pH 4.0 with H3PO4.

Mobile Phase A : (70: 30) Buffer: Acetonitrile

Mobile Phase B : (20: 80) Buffer: Acetonitrile

Mix well, filtered and degassed the Mobile Phase.

Gradient Programme:

Time in min	% of A	% of B
0.01	60	40
5.00	60	40
30.00	0	100
50.00	0	100
52.00	60	40
60.00	60	40

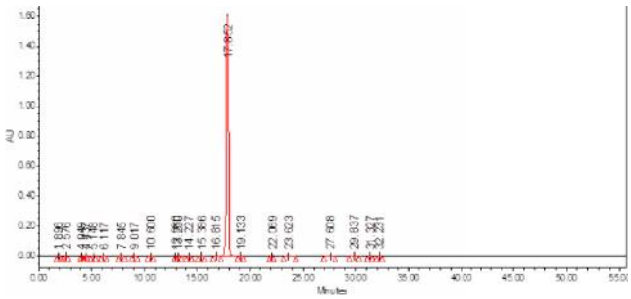

Fig 5:RIM Pharm Chromatogram

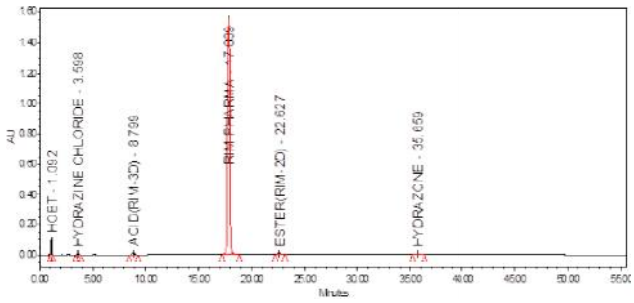

Fig 6:RIM Pharma Spiked Chromatogram

Observation: In the above conditions unknown impurity before main peak is separating well.Ester and Hydrazine impurities are eluting in this method and Acid (RIM 3D) is separating well.

4. Conclusion

Analytical method development for theidentification of related substances and impurities of Rimonabent by using high performance liquid Chromatography. For the identification purpose four chromatographic trials were conducted to the different chromatographic conditions. First three trials the substances are not elueted properly. The substances and impurities are clearly separated in final trial. So, the method was successfully developed for estimation of related substances and impurities of Rimonabent.

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