A RESEARCH OF DEVELOPMENTAL ANALYSIS OF HPLC METHOD IN MOXONIDINE HYDROCHLORIDE

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ABSTRACT

This Scientific paper consists of the information of HPLC new method development and Validation as per ICH Guidelines. It also explains importance of HPLC method development and types of HPLC columns. In this paper new binger clearly understand how new method development carried out and what are the ICH guidelines regarding any new method development by HPLC. A simple, precise, specific sensitive and accurate high performance liquid chromatographic (HPLC) method was developed for the determination of Moxonidine in tablet dosage form. This method then applied on formulations as its application. It will help to budding students for new method development and it's validation A simple, sensitive, precise and rapid high performance liquid chromatography (HPLC) method was developed and validated for simultaneous estimation of Moxonidine in bulk drug and tablet dosage forms. The separation was achieved by using Qualisil BDS C18 (25cm X 4mm, 5μm) as stationary phase and mobile phase consists of Buffer: Acetonitrile (864 V: 136V) with a flow rate of 1.2 ml/min. Analysis was performed at ambient temperature with detection at 230 nm. The retention times of Moxonidine were found to be 2.6 ± 2 min. The percentage recoveries of Moxonidine at assay level were found to be in the range of 9.0% to 103.0% The method was validated in the terms of reproducibility and recovery studies. Moxonidine. The method precision for the determination of assay was below 2.0% RSD. A rapid and precise High Performance Liquid Chromatographic method has been developed for the validated of Moxonidine, in its pure form as well as in tablet dosage form. The developed method was validated for parameters

like system suitability, specificity, linearity, accuracy, precision, ruggedness and robustness as per ICH guidelines and the results were found to be within the limits. So it can be used for the routine quality control of Moxonidine in bulk sample and tablet dosage forms. The validation of the proposed method was also performed considering selectivity, linearity, accuracy, precision, limit of detection and limit of quantification, and the results indicated that the method fulfilled all required criteria. The method was successfully applied to the analysis of commercial tablets. The proposed method was found to be accurate, reproducible and economical for routine analysis of Moxonidine in tablet dosage form. The system suitability parameters were calculated to ascertain the suitability of the proposed method in mobile phase at 230nm as a detection wavelength. Theoretical plates HETP, Tailing factor and Resolution were shown to be well within the acceptance criteria.

The developed HPLC method was then applied for the simultaneous estimation of Moxonidine in marketed tablet formulation. The % assay of Moxonidine by proposed method was obtained in the range of 99.0%-105 %respectively, which was well within the acceptance criteria limit of 90-110% indicating that the method can be applied for simultaneous determination of Moxonidine in marketed tablet formulation. Hence, the developed HPLC method results shows to be accurate, precise, linear, robust, rugged and specific.

The proposed method is simple, accurate, cost effective, less time consuming and the analysis proved that the method is reproducible and efficient for the simultaneous estimation of Moxonidine as bulk drugs and combined dosage forms without any interference from the excipients. The developed method could be conveniently adopted for routine analysis in quality control laboratories.

KEYWORDS: Moxonidine, British Pharmacopoeia, Monograph, High performance liquid chromatographic (HPLC), Assay, Analytical Method Validation.

INTRODUCTION

Although there are many analyzes for this drug, this process (or) analysis is newly designed. This analysis gives us everything we need i.e. quality, quantity, estimation, precision etc. of a drug. It is analyzed by british pharmacopoeia and only this method is available. This analysis is done only by spectroscopy but this research shows that it can also be done by hplc analysis. Purity, quality and other functions of the drug are given by doing this method.

Moxonidine

It is a new-generation centrally acting antihypertensive drug approved for the treatment of mild to moderate essential hypertension. It is suggested to be effective in cases where other agents such as thiazides, beta-blockers, ACE inhibitors, and calcium channel blockers are not appropriate or irresponsive. As well, moxonidine has been shown to present blood pressure-independent beneficial effects on insulin resistance syndrome.

Moxonidine is the newest, second generation, centrally acting antihypertensive agent. It has selective agonist activity at imidazoline I1 receptors and less adverse effects than the other centrally acting sympatholytic drugs. It is a selective agonist at the imidazoline receptor subtype.

1. Moxonidine causes a decrease in sympathetic nervous system activity and therefore a decrease in blood pressure. It also used with ACE inhibitors, diuretics and calcium channel blockers. In addition it demonstrates favorable effects on parameters of the insulin resistance syndrome, apparently independent of blood pressure reduction.

2. Moxonidine is an imidazoline/α-2 receptor agonist used to treat hypertension, especially in cases where ACE inhibitors, β-blockers, calcium channel blockers, and thiazides are not appropriate or provide inadequate blood pressure control.

This fact authorizes the frequent use of moxonidine in clinical practice, as monotherapy or in combination with other antihypertensive agents. Also, moxonidine is used in the treatment of obese patients with metabolic syndrome, because this antihypertensive agent reduces leptin levels in plasma and reduces weight in obese patients, through the action on the Sympathetic Nervous System (SNS).

Second generation centrally acting antihypertensive agents, like moxonidine, in the management of hypertension in obese. Blood Pressure (BP) measurements were done by mercury sphygmomanometer according to the European Hypertension Society guidelines. The dietary intake and the exercise of the patients, was the same before and after the treatment. Patients with diabetes or other chronic diseases were excluded. Statistical analysis was made by t student test (SPSS) and the level of statistical significance was defined at $p<0.05$.

Conclusively, women either obese or normal weight, seems to respond better to antihypertensive treatment with moxonidine, with a reduction of SBP and DBP which may reflect the greater stimulation of the SNS in women4 before treatment and contribution to the increase of blood pressure, as well as a more beneficial effect on BW reduction in females.

HPLC (Svetlana, 2012) HPLC, Mass Spectrometry and UPLC methods are studied for the determination of Monoxidine and RP-HPLC and other methods used. Hence the present study designed to perform estimation of Moxonidine. The aim of study is to developmental analysis of moxonidine in using HPLC method.[2],[3]

Drug profile
Chemical name: Moxonidine hydrochloride
Chemical formula: $C_9H_{12}ClN_5O$

Chemical structure
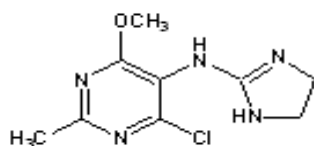
Contraindication

It is contraindicated if there has been a past history of angioedema; heart conduction disorders (e.g. sick sinus syndrome, second- or third-degree heart block); bradycardia; severe heart failure or coronary artery disease. Also: Raynaud's syndrome, intermittent claudication, epilepsy, depression, Parkinson's disease, glaucoma. Use in pregnancy is discouraged. Moxonidine passes into breast milk.

Moxonidine should be avoided in patients with moderate to severe renal impairment. Abrupt discontinuation of the drug should also be avoided. If concomitant treatment with a beta blocker has to be stopped, the beta blocker should be discontinued first, then moxonidine after a few days. Alcohol may potentiate the hypotensive effects of Moxonidine.

Mechanism of action

Moxonidine is a selective agonist at the imidazoline receptor subtype 1 (I_1). This receptor subtype is found in both the rostral ventro-lateral pressor and ventromedial depressor areas of the medulla oblongata. Moxonidine therefore causes a decrease in sympathetic nervous system activity and, therefore, a decrease in blood pressure.

Compared to the older central-acting antihypertensives, moxonidine binds with much greater affinity to the imidazoline I_1-receptor than to the α_2-receptor. In contrast, clonidine binds to both receptors with near equal affinity. Moxonidine has an affinity for I_1 that is 33 times greater than α_2, compared to clonidine which is only four times greater.

In addition, moxonidine may also promote sodium excretion, improve insulin resistance and glucose tolerance and protect against hypertensive target organ damage, such as kidney disease and cardiack.

Effects of insulin resistance

In all animal models of insulin resistance, moxonidine had striking effects on the development of insulin resistance, hyperinsulinaemia and impaired glucose homeostasis. Given the importance of insulin resistance as a risk factor for cardiovascular disease, it is of considerable relevance that it has been shown to improve insulin sensitivity.

Indication: For the treatment of mild to moderate essential or primary hypertension. Effective as most first-line antihypertensives when used as monotherapy

Pharmacodynamics: Antihypertensive agent whose site of action is the Central Nervous System (CNS), specifically involving interactions with I1- imidazoline and alpha-2-adrenergic rececptors within the rostral ventrolateral medulla (RSV).

Absorption: 90% of an oral dose is absorbed with negligible interference from food intake or first pass metabolism, resulting in a high bioavailability of 88%.

Volume of distribution: 1.8±0.4L/kg

Protein binding: About 10% of moxonidine is bound to plasma proteins.

Half-life: Plasma elimination half life is 2.2 - 2.3 hours while renal elimination half life is 2.6-2.8 hours.

Toxicity

- Contraindicated due to known hypersensitivity to an ingredient (Physiotens tablets contain lactose), heart failure, severe renal impairment, < 16 years old, >75 years old, bradycardia, severe bradyarrhythmia, sick sinus syndrome, second or third degree atrioventricular block, malignant arrhythmias.
- Used with caution in patients with history of severe coronary artery disease (CAD), unstable angina, angioneurotic edema.
- Pregnancy Category B3: Avoid use during pregnancy (inadequate data in pregnant woman) and lactation (maternal blood stream transfer to breast milk shown) unless benefit clearly justifies risk.
- Lack of specific therapeutic experience in cases of intermittent claudication, Raynaud's disease, Parkinson's disease, epileptic disorders, gluacoma, and depression suggest moxonidine should not be used in such instances Carcinogenicity and genotoxicity does not appear significant.
- Concurrent administration of other hypotensives or sedative and hypnotics can enhance the hypotensive effect and intensify sedation respectively.
- Avoid concurrent Tricyclic Antidepressant (TCA) use to avoid reduction of monoxidine efficacy.
- Generally well tolerated with dry mouth and headache the most common adverse effects Symptoms of overdose correlate with pharmacodynamic

Properties: Hypotension, sedation, orthostatic dysregulation, bradycardia, dry mouth with no specific counter-treatment known.

Summary of dosages characteristics

Qualitative and Quantitative composition

- Each tablet contains 0.2 mg moxonidine.
- Each tablet contains 0.3 mg moxonidine.
- Excipient with known effect: Microcrystalline cellulosse powder 102 ,Starch, Talc, Magnesium stearate, Sodium Starch glycolate.

Clinical particulars

Therapeutic indications

Mild to moderate essential hypertension.

Posology and Method of administration

Posology.

Adults

➤ Treatment must be instituted with the lowest dosage of Moxonidine. This means a daily dose of 0.2 mg moxonidine in the morning.

➤ If the therapeutic effect is insufficient, the dose can be increased after three weeks to 0.4 mg.

➤ This dose can be given as a single dose (to be taken in the morning) or as a divided daily dose (morning and evening).

➤ If the results are still insufficient after a further three weeks, the dosage can be increased further to a maximum of 0.6 mg given divided in the morning and evening. A single dose of 0.4 mg

➤ Moxonidine and a daily dose of 0.6 mg Moxonidine should not be exceeded.

Paediatric population

➤ Moxonidine should not be given to children and adolescents under 16 years of age as insufficient therapeutic data are available for this.

Elderly

➤ Provided that renal function is not impaired, dosage recommendation is the same as for adults.

➤ The treatment should not be stopped abruptly, but withdrawn over a period of two weeks.

Method of administration

➤ As concomitant ingestion of food does not affect the pharmacokinetics of moxonidine, Moxonidine can be taken before, during or after meals. The tablets should be taken with sufficient fluid.

Contraindications

o Hypersensitivity to the active substance or to any of the excipients listed.

o sick sinus syndrome or sino-atrial block

o bradycardia (Resting HR < 50 beats/minute)

o AV-block 2nd or 3rd degree

o cardiac insufficiency

Special Warnings and Precautions for use

➢ Cases of varying degrees of AV block have been reported in the post-marketing setting in patients undergoing moxonidine treatment. Based on these case reports, the causative role of moxonidine in delaying atrioventricular conduction cannot be completely ruled out.

➢ Therefore, caution is recommended when treating patients with a possible predisposition to developing an AV block.

➢ When moxonidine is used in patients with 1st degree AV block special care should be exercised to avoid bradycardia. Moxonidine must not be used in higher degree AV blocks. When moxonidine is used in patients with severe coronary artery disease or unstable angina pectoris special care should be exercised due to the fact that there is limited experience in this patient population.

➢ Caution is advised in the administration of moxonidine to patients with renal impairment as moxonidine is excreted primarily via kidney. In these patients careful titration of the dose is recommended, especially at the start of therapy.

➢ Dosing should be initiated with 0.2 mg daily and can be increased to a maximum of 0.4 mg daily for patients with moderate renal impairment (GFR >30 ml/min but <60 ml/min) and to a maximum of 0.3 mg daily for patients with severe renal impairment (GFR <30 ml/min), if clinically indicated and well tolerated.

➢ If moxonidine is used in combination with a beta-blocker and both treatments have to be discontinued, the beta-blocker should be discontinued first, and then moxonidine after a few days.

➢ So far, no rebound-effect has been observed on the blood pressure after discontinuing the treatment with moxonidine. However, an abrupt discontinuance of the moxonidine treatment is not advisable; instead the dose should be reduced gradually over a period of two weeks.

➢ The elderly population may be more susceptible to the CV effects of blood pressure lowering drugs. Therefore therapy should be started with the lowest dose and dose increments should be introduced with caution to prevent the serious consequences these reactions may lead to.

➢ Patients with rare hereditary problems of galactose intolerance, Lapp lactase deficiency or glucose-galactose malabsorption should not take this medicine.

Interaction with other medicinal Products and Other forms of interaction

➤ Concomitant administration of moxonidine and other antihypertensives agents result in an additive effect.

➤ Since tricyclic antidepressants may reduce the effectiveness of centrally acting antihypertensive agents, it is not recommended that tricyclic antidepressants are co-administered with moxonidine.

➤ Moxonidine can potentiate the sedative effect of tricyclic anti-depressants (avoid coprescribing), tranquillisers, alcohol, sedatives and hypnotics.

➤ Moxonidine moderately augmented the impaired performance in cognitive functions in subjects receiving lorazepam. Moxonidine may enhance the sedative effect of benzodiazepines when administered concomitantly.

➤ Moxonidine is excreted through tubular excretion. Interaction with other agents that are excreted through tubular excretion cannot be excluded.

Fertility, Pregnancy and Lactation

Pregnancy

There are no adequate data from use of moxonidine in pregnant women. Studies in animals have shown embryo-toxicological effects (see section 5.3). The potential risk for humans is unknown.

Moxonidine should not be used during pregnancy unless clearly necessary.

Lactation

Moxonidine is secreted in breast milk and should therefore not be used during breastfeeding. If therapy with moxonidine is considered absolutely necessary, the breastfeeding shall be stopped.

Effects on ability to Drive and Use machines

No studies on the effects on the ability to drive and use machines have been performed. Somnolence and dizziness have been reported. This should be borne in mind when performing these tasks.

Undesirable effects

Summary of the safety profile

Most frequent side effects reported by those taking moxonidine include dry mouth, dizziness,

asthenia and somnolence. These symptoms often decrease after the first few weeks of treatment.

Reporting of suspected adverse reactions

Reporting suspected adverse reactions after authorisation of the medicinal product is important.

It allows continued monitoring of the benefit/risk balance of the medicinal product. Healthcare professionals are asked to report any suspected adverse reactions via the national reporting system listed in Appendix V.

Overdose

Symptoms of overdose

In the few cases of overdose that have been reported, a dose of 19.6 mg was ingested acutely without fatality. Signs and symptoms reported included: headache, sedation, somnolence, hypotension, dizziness, asthenia, bradycardia, dry mouth, vomiting, fatigue and upper abdominal pain. In case of a severe overdose close monitoring of especially consciousness disturbances and respiratory depression is recommended.

In addition, based on a few high dose studies in animals, transient hypertension, tachycardia, and hyperglycaemia may also occur.

Treatment of overdose

➢ No specific antidote is known. In case of hypotension, circulatory support such as fluids and dopamine administration may be considered. Bradycardia may be treated with atropine.

➢ Alpha-Receptor antagonists may diminish or abolish the paradoxical hypertensive effects of a moxonidine overdose.

Pharmacological properties

Pharmacodynamic properties

➢ In various animal models it has been shown that moxonidine has a strongly hypotensive effect.

➢ Available experimental data indicate that the site of action of moxonidine is located in the central nervous system (CNS).

➢ In the brain stem, moxonidine binds selectively to I1-imidazoline receptors. These imidazolinesensitive receptors are predominantly found in the rostral ventrolateral medulla, an area which plays an important role in central control of the sympathetic nervous system. The effect of this interaction with these I1-imidazoline receptors appears to be a reduction in the activity of the sympathetic nerves. This has been demonstrated for cardiac, splanchnic and renal sympathetic nerves.

➢ Moxonidine differs from other centrally acting hypertensives in the fact that it has only a weak affinity for the central α2-adrenergic receptors compared to the affinity for I1-imidazoline receptors.

➢ Alpha2-adrenergic receptors are considered to be the intermediate pathway that causes sedation and dry mouth, the most commonly observed undesirable effects of centrally acting antihypertensives.

➢ Mean systolic and diastolic blood pressure is reduced both at rest and during exercise.

➢ The effects of moxonidine on mortality and cardiovascular morbidity are currently unknown.

Pharmacokinetic properties

Absorption

➢ Moxonidine is rapidly absorbed after oral administration. In humans, approximately 90% of an oral dose is absorbed. Ingestion of food has no effect on the pharmacokinetics of moxonidine. There is no first-pass metabolism and bioavailability is 88 %.

Distribution

➢ Only about 7% of moxonidine is bound to human plasma proteins (Vdss = 1.8 $\square$ 0.4 l/kg). Peak plasma levels of moxonidine are reached 30-180 minutes after administration of a film-coated tablet.

Biotransformation

➢ Moxonidine is 10-20% metabolised, predominantly to 4,5-dehydromoxonidine and to an aminomethanamidine derivative by opening of the imidazoline ring. The hypotensive effect of 4,5-dehydromoxonidine is only 1/10, and that of the aminomethanamidine derivative less than 1/100, of that of moxonidine.

Elimination

➢ Moxonidine and its metabolites are almost entirely eliminated via the kidney. More than 90% of the dose is eliminated in the first 24 hours via the kidney, while approximately 1% is eliminated in the faeces. The cumulative excretion of unchanged moxonidine is approximately 50-75%.

➢ The mean plasma elimination half life is 2.2-2.3 hours and the renal half-life 2.6-2.8 hours.

➢ In patients with moderately impaired renal function (GFR 30-60 ml/min), the AUC increased by 85% and the clearance reduced by 52%. The dose must be adapted in these patients so that the maximum daily dose is not more than 0.4 mg and the maximum single dose is 0.2 mg.

➢ In patients with severely impaired renal function (GFR <30 ml) the clearance is reduced by 68 % and the elimination half live is prolonged up to 7 hours. In these patients moxonidine dosing should be initiated with 0.2 mg daily and can be increased to a maximum of 0.3 mg daily, if clinically indicated and well tolerated.

Preclinical safety data

➢ Preclinical data reveal no special hazard for humans based on conventional studies of repeated toxicity, genotoxicity and carcinogenic potential.

➢ Reproductive toxicity studies revealed no effects on fertility and no teratogenic potential.

➢ Embryotoxic effects were seen in rats at dosages above 3 mg/kg/d and in rabbits at dosages above 0.7 mg/kg/d. In a perinatal and postnatal study in rats the development as well as the viability of the offspring was affected in dosages above 1 mg/kg/d.[13],[14],15],[16]

Pharmaceutical particulars

List of excipients

Tablet core

Microcrystalline cellulosse powder 102

Starch

Talc

Magnesium stearate

Sodium Starch glycolate

Film-coating

Protectab HP 1 Organic

titanium dioxide (E171)

PEG 6000

Shelf life: 2 years

Special precautions for storage

➢ Do not store above 30°C& Store in the original container in order to protect from light.

MATERIALS AND METHODS

Chemicals and Reagents

Moxonidine, Acetonitrile (HPLC grade), methanol and reagents of analytical grade were procured from India. HPLC grade water is collected from Milli-Q3 water purifier system. Class A apparatus were used throughout the experiment. The analysis are procured from british pharmacopeia.

Instrumental and Chromatographic system

HPLC system

Shimadzu, LC-2030C model attached with pump, degasser, auto sampler, Ultra-violet detector.

Chromatographic column

Mobile phase	**:** Buffer: ACN (864V: 136V)
Column	**:** C_{18} (25cm X 4mm, 5µm)
Flow rate	**:** 1.2 mL/min
Wavelength	**:** 230nm
Injection volume	**:** 20µL
Run time	**:** 10 minutes
Column temperature	**:** 40°C

Buffer preparation

Dissolve accurately *3.48 gm* of Sodium pentane sulfonate, dissolved in 1000mL with water and adjusted the pH to 3.5 with ortho-sulphuric acid.

Mobile phase: Buffer: Acetonitrile (864 V: 136V)

Preparation of Standard and Sample solutions

Standard solution preparation

Weigh accurately 10 mg of Moxonidine working Reference Standard in 100ml volumetric flask add Methanol and sonicate to 15 minutes to dissolve make up to the mark with Methanol.

Pipette out 2 ml of the above solution in 20 ml of volumetric flask add to Methanol.

Sample solution preparation

Weigh accurately equivalent to 1 mg of sample in 100 ml volumetric flask add Methanol and sonicate to 15 minutes to dissolve make up to the mark with Methanol.

Determination of wavelength range

Wavelength for detection (UV detector) was selected by injecting the solution consists of Moxonidine (10μg/ml) to SHIMADZU- LC-2030C instrument. The overlay spectrum shows isobestic point at 230nm which was selected as wavelength of detection, the overlay spectrum.

Determination of retention time

The retention time for Moxonidine were determined individually and in combination by injecting 20μl of working standard solutions at 1.2 ml/min into the chromatograph and UV detected at 230nm. Retention time was observed; chromatogram was recorded.[1],[19]

RESULTS AND DISCUSSION

Determination HPLC condition Chromatograms of blank, assay standard, moxonidine API, moxonidine 0.2mg tablets & moxonidine 0.3mg tablets. The chromatograms of UV-HPLC revealed that the compounds peaks are distinct and clear without any placebo interference thereby highlighting the efficiency of both sensitivity and selectivity of the optimized protocol. The runtime of the procedure is found to be exactly 10 minutes and the peaks were of good shape and completely resolved.

A simple, rapid, isocratic, stability-indicating, high-performance liquid chromatographic (HPLC) method was developed and validated for the routine analysis of moxonidine in the presence of its degradation products in active pharmaceutical ingredient and pharmaceutical dosage forms. Forced degradation studies were performed according to the guidance of International Conference for Harmonization and were used to evaluate moxonidine intrinsic

stability. The drug was subjected to acid, neutral and base hydrolysis as well as to oxidative, thermal and photolytic decomposition in both solution and solid state. The drug appeared to be unstable towards acid and base hydrolysis. To achieve desirable conditions for HPLC analysis, the method development was done with the assistance of experimental design and multivariate optimization methodology function. Stress samples were analyzed according to the experimental for fractional factorial screening design and optimization design. The chromatographic separation was achieved on a C_{18} (25cm X 4mm, 5µm) with the mobile phase consisting of Buffer: ACN (864V: 136V) (pH to 3.5) mixture pumped at a flow rate of 20 µL min−1 and detection wavelength of 230 nm. The analysis of the proposed method was also performed considering, assay, limit of detection and limit of quantification, and the results indicated that the method fulfilled all required criteria. The method was successfully applied to the analysis of commercial tablets of 0.3 mg & 0.2 mg dosage form.

Raw material analysis of moxonidine (Active pharmaceutical analysis)

Name of the sample: Moxonidine BP

Description: White or almost white powder

Solubility: Very slightly soluble in water, Sparingly soluble in Methanol, Slightly soluble in methylene chloride, Very slightly soluble in acetonitrile.

OBSERVATION

1. **Sulphated ash:** NMT 0.1 %, Determination on 1.0 g.

Calculation

Weight of the crucible + Sample before ignition g **(V$_2$): 33.3815 g**

Weight of the empty crucible in g **(V$_1$): 32.3614 g**

Weight of the crucible + Residue after ignition g (V$_3$): 32.3606 g

$$\frac{V_3 - V_1}{V_2 - V_1} \times 100 = \frac{0.0008}{1.0201} \times 100 = 0.07 \%$$

2. **Loss on drying:** NMT 0.5%, determination on 1.000g By drying in an oven at 105^0C for 3 Hr.

Calculation

Weight of the bottle + Sample before Drying in gm **(V$_2$): 36.2600 g**

Weight of the empty bottle in gm (V$_1$): 34.6349 g

Weight of the bottle + Residue after in gm (V$_3$) : 36.2528 g

$$\frac{V_2 - V_3}{V_2 - V_1} \times 100 = \frac{0.0072}{1.6251} \times 100 = 0.44\,\%$$

3. Assay (Limit 97.5% -102.00%)

Buffer preparation

Dissolve accurately 3.48 gm of sodium pentane sulfonate, dissolved in 1000 ml with water and adjusted the pH to 3.5 with suphuric acid.

Mobile phase: Buffer : Acetonitrile (864 V : 136 V)

Parameters

Mobile Phase   : Buffer: ACN (864V: 136V)

Column   : C_{18} (25cm X 4mm, 5µm)

Flow rate   : 1.2 mL/min

Wavelength   : 230nm

Injection volume   : 20µL

Run time   : 10 minutes

Column temperature : 40°C

Calculation

1. Contains Moxonidine BP

$$= \frac{\text{Spl Area}}{\text{Std Area}} \times \frac{\text{Std wt}}{100} \times \frac{2}{20} \times \frac{100}{\text{spl wt}} \times \frac{20}{2} \times \frac{\text{Std.potency}}{100} \times 100$$

Working standard weight: 0.0101

Sample weight = 0.0102

$$= \frac{241340}{240218} \times \frac{0.0101}{100} \times \frac{2}{20} \times \frac{100}{0.0102} \times \frac{20}{2} \times \frac{99.35}{100} \times 100$$

= 98.84%

Sample Information

Sample Name   : Moxonidine
Sample ID   : Std 001
Data Filename   : lc_301120230002.lcd
Method Filename   : Moxonidine Assay.lcm
Batch Filename   : lc_301120230001.lcb
Vial #   : 1-2   Sample Type   : Unknown
Injection Volume   : 20 uL
Date Acquired   : 30-11-2023 09:21:44   Acquired by   : System Administrator
Date Processed   : 30-11-2023 09:41:13   Processed by   : System Administrator

Summary(Compound)
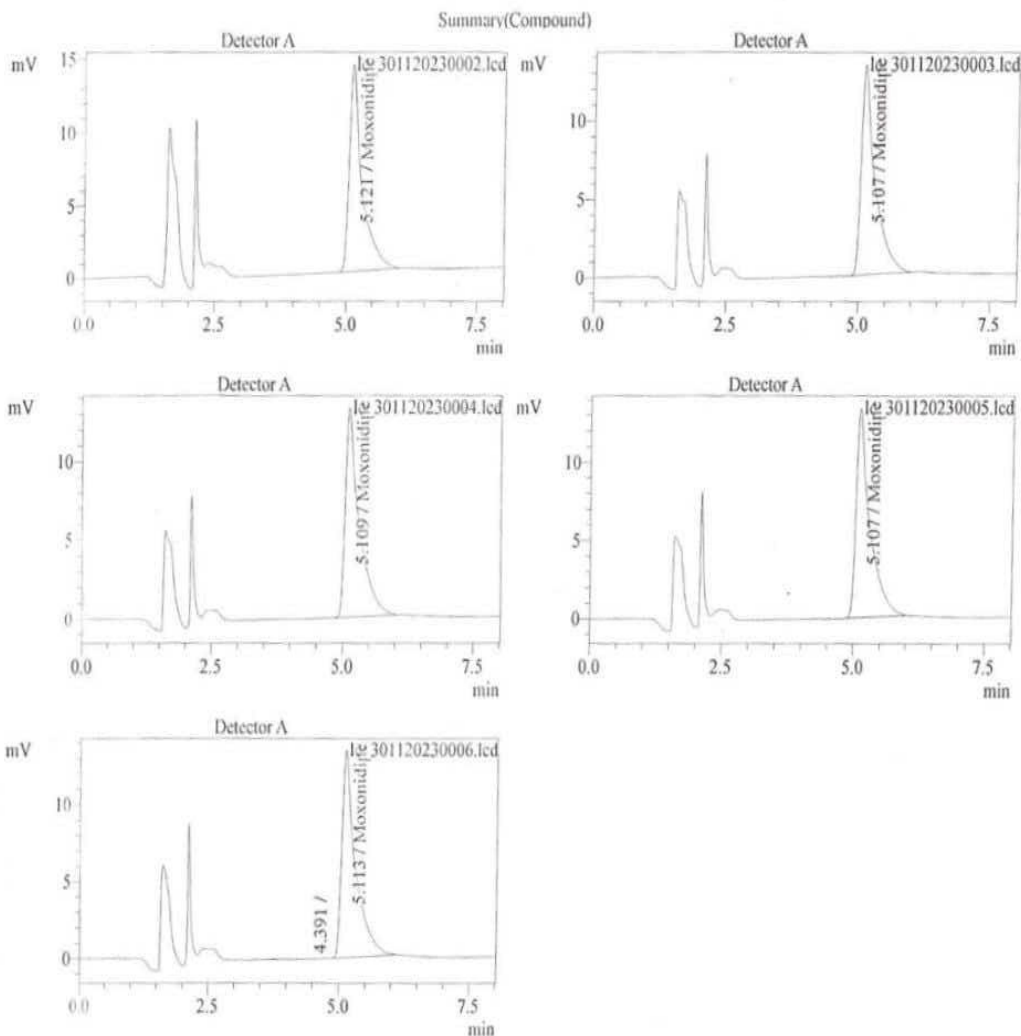
<< Detector A >>
ID#1 Compound Name: Moxonidine

Title	Sample Name	Sample ID	Ret. Time	Area	Height	Conc.	Tailing Factor
lc 301120230002.lcd	Moxonidine	Std 001	5.121	237812	14158	0.000	2.306
lc 301120230003.lcd	Moxonidine	Std 002	5.107	241493	13317	0.000	2.471
lc 301120230004.lcd	Moxonidine	Std 003	5.109	241979	13256	0.000	2.497
lc 301120230005.lcd	Moxonidine	Std 004	5.107	240821	13375	0.000	2.492
lc 301120230006.lcd	Moxonidine	Std 005	5.113	238984	13390	0.000	2.572
Average			5.111	240218	13499	0.000	2.468
%RSD			0.111	0.733	2.756	0.000	3.978
Maximum			5.121	241979	14158	0.000	2.572
Minimum			5.107	237812	13256	0.000	2.306
Standard Deviation			0.006	1761	372	0.000	0.098

E:\DATA\2023\NOV-2023\30-2023\lc_301120230002.lcd

Sample Information

Sample Name   : Moxonidine (RM)
Sample ID   : MOX0010623
Data Filename   : lc_301120230007.lcd
Method Filename : Moxonidine Assay.lcm
Batch Filename   : lc_301120230001.lcb
Vial #   : 1-3
Injection Volume : 20 uL
Date Acquired   : 30-11-2023 10:38:11
Date Processed   : 30-11-2023 10:56:25

Sample Type   : Unknown

Acquired by   : System Administrator
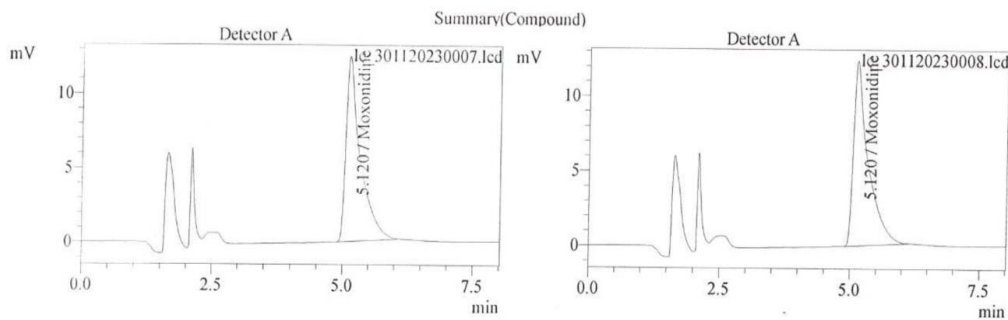Processed by   : System Administrator

Summary(Compound)

<< Detector A >>
ID#1 Compound Name: Moxonidine

Title	Sample Name	Sample ID	Ret. Time	Area	Height	Conc.	Tailing Factor
lc_301120230007.lcd	Moxonidine (I	MOX0010623	5.120	241884	12477	0.000	2.634
lc_301120230008.lcd	Moxonidine (I	MOX0010623	5.120	240797	12374	0.000	2.652
Average			5.120	241340	12426	0.000	2.643
%RSD			0.001	0.318	0.587	0.000	0.493
Maximum			5.120	241884	12477	0.000	2.652
Minimum			5.120	240797	12374	0.000	2.634
Standard Deviation			0.000	768	73	0.000	0.013

Theoretical Plates/meter(USP)	Resolution(USP)
15764	--
16361	--
16062	--
2.629	0.000
16361	--
15764	--
422	--

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Moxonidine 0.3 mg coated tablets analysis

Test method: IHS / IP

Description: Take few coated tablets and examine visually, note down the observation.

Observation: White color circular shaped slightly biconvex film coated tablets having plain on both side of the tablets.

Identification: Retention time of principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Weight variation

Weight of 20 tablets: 2.8639 g

Average weight of a tablets: 0.1432 g

Uniformity of weights: (7.5 %) 0.1322 g – 0.1537 g

Weigh 20 tablets selected at random and determine the individual weight.

0.1430	0.1418	0.1456	0.1450	0.1406
0.1460	0.1408	0.1428	0.1460	0.1432
0.1426	0.1414	0.1445	0.1435	0.1456
0.1444	0.1436	0.1433	0.1412	0.1433

Disintegration time: (Limit not more than 15 minutes)

Place 1 dosage unit in each of the six tubes of the basket and add a disk in all six tubes of the basket. Operate the apparatus, Using water as the immersion fluid, maintained at 37^0 C (+ or) 2^0C.Watch the disintegration of baskets, after disintegration of tablets from all baskets lift the basket from the fluid, and check whether all of the tablets have disintegration completely, if so note down the time 1min: 45 sec.

Assay: (90.0 % -110.0 %)

Calculation

Each film coated tablets contains Moxonidine 0.3 mg

$$= \frac{\text{Spl Area}}{\text{Std Area}} \times \frac{\text{Std wt}}{100} \times \frac{2}{20} \times \frac{100}{\text{spl wt}} \times \frac{\text{Std.potency}}{100} \times \text{Average wt of tablets} \times 1000$$
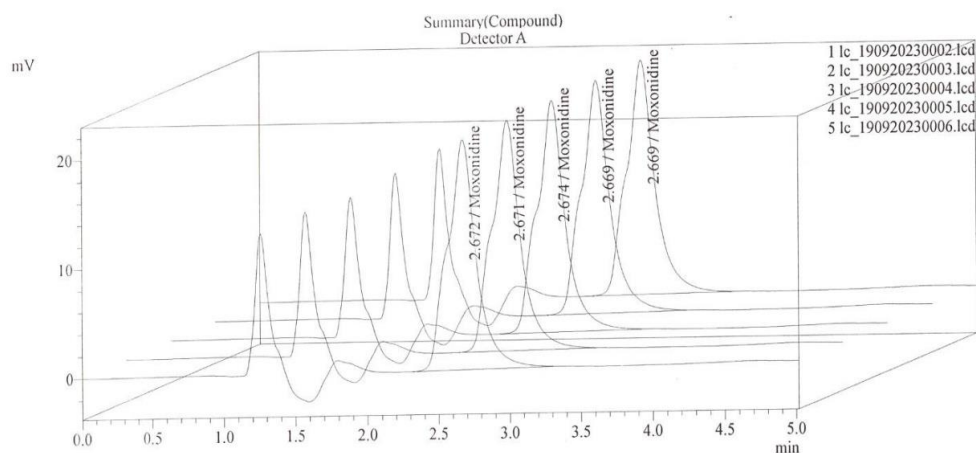
Working standard weight: 0.0102

Sample weight = 0.4831

$$= \frac{301624}{296691} \times \frac{0.0102}{100} \times \frac{2}{20} \times \frac{100}{0.4831} \times \frac{99.35}{100} \times 143.2$$

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Sample Information

Sample Name   : Moxonidine
Sample ID   : Std001
Data Filename   : lc_190920230002.lcd
Method Filename : Moxonidine Assay.lcm
Batch Filename   : lc_190920230001.lcb
Vial #   : 1-2
Injection Volume : 20 uL
Date Acquired   : 19-09-2023 10:46:40
Date Processed   : 19-09-2023 10:54:09

Sample Type   : Unknown

Acquired by   : System Administrator
Processed by   : System Administrator

Summary(Compound)
Detector A

1 lc_190920230002.lcd
2 lc_190920230003.lcd
3 lc_190920230004.lcd
4 lc_190920230005.lcd
5 lc_190920230006.lcd

<< Detector A >>
ID#1 Compound Name: Moxonidine

Title	Sample Name	Sample ID	Ret. Time	Area	Height	Conc.
lc_190920230002.lcd	Moxonidine	Std001	2.669	296173	21481	0.000
lc_190920230003.lcd	Moxonidine	Std002	2.669	297009	21379	0.000
lc_190920230004.lcd	Moxonidine	Std003	2.674	296817	21270	0.000
lc_190920230005.lcd	Moxonidine	Std004	2.671	296587	21122	0.000
lc_190920230006.lcd	Moxonidine	Std005	2.672	296869	21041	0.000
Average			2.671	296691	21259	0.000
%RSD			0.072	0.110	0.848	0.000
Maximum			2.674	297009	21481	0.000
Minimum			2.669	296173	21041	0.000
Standard Deviation			0.002	327	180	0.000

Number of Theoretical Plate(USP)	Tailing Factor
987	1.188
971	1.190
1015	1.149
958	1.163
965	1.157
979	1.170
2.303	1.611
1015	1.190
958	1.149
23	0.019

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Sample Information

Sample Name   : Moxonidine (Unsym-0.3)
Sample ID   : BT587
Data Filename   : lc_190920230009.lcd
Method Filename : Moxonidine Assay.lcm
Batch Filename : lc_190920230001.lcb
Vial #   : 1-4   Sample Type   : Unknown
Injection Volume : 20 uL
Date Acquired   : 19-09-2023 11:24:52   Acquired by   : System Administrator
Date Processed : 19-09-2023 11:29:59   Processed by   : System Administrator

Summary(Compound)
Detector A
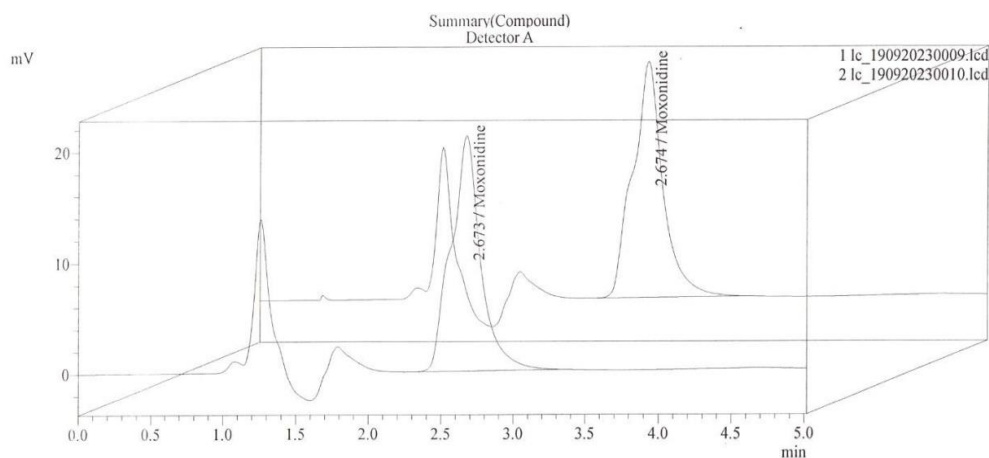
<< Detector A >>
ID#1 Compound Name: Moxonidine

Title	Sample Name	Sample ID	Ret. Time	Area	Height	Conc.
lc_190920230009.d	Moxonidine (l	BT587	2.674	302223	21276	0.000
lc_190920230010.d	Moxonidine (l	BT587	2.673	301045	21219	0.000
Average			2.673	301634	21248	0.000
%RSD			0.021	0.276	0.191	0.000
Maximum			2.674	302223	21276	0.000
Minimum			2.673	301045	21219	0.000
Standard Deviation			0.001	833	41	0.000

Number of Theoretical Plate(USP)	Tailing Factor
992	1.173
1003	1.176
997	1.174
0.829	0.191
1003	1.176
992	1.173
8	0.002

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Moxonidine 0.2 mg coated tablets analysis

Test method: IHS / IP

Description: Take few coated tablets and examine visually, note down the observation.

Observation: White color circular shaped slightly biconvex film coated tablets having plain on both side of the tablets.

Identification: Retention time of principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Weight variation

Weight of 20 tablets: 2.8813 g

Average weight of a tablets: 0.1441 g

Uniformity of weights: (7.5 %) 0.1322 g – 0.1537 g

Weigh 20 tablets selected at random and determine the individual weight.

0.1432	0.1443	0.1449	0.1429	0.1430
0.1455	0.1450	0.1432	0.1463	0.1461
0.1412	0.1446	0.1443	0.1428	0.1454
0.1445	0.1455	0.1447	0.1437	0.1442

Disintegration time: (Limit not more than 15 minutes)

Place 1 dosage unit in each of the six tubes of the basket and add a disk in all six tubes of the basket. Operate the apparatus, Using water as the immersion fluid, maintained at 37^0C (+or-) 2^0C. Watch the disintegration of baskets, after disintegration of tablets from all baskets lift the basket from the fluid, and check whether all of the tablets have disintegration completely, if so note down the time 1 min : 33 sec.

Assay: (90.0 % -110.0 %)

Calculation

Each film coated tablets contains Moxonidine 0.3 mg

$$= \frac{Spl\ Area}{Std\ Area} \times \frac{Std\ wt}{100} \times \frac{2}{20} \times \frac{100}{spl\ wt} \times \frac{Std.potency}{100} \times Average\ wt\ of\ tablets \times 1000$$

Working standard weight: 0.0102

Sample weight = 0.7382

$$= \frac{310196}{296691} \times \frac{0.0102}{100} \times \frac{2}{20} \times \frac{100}{0.7382} \times \frac{99.35}{100} \times 144.1$$

= 0.207 mg (103.41%)

Sample Information

Sample Name	: Moxonidine		
Sample ID	: Std001		
Data Filename	: lc_190920230002.lcd		
Method Filename	: Moxonidine Assay.lcm		
Batch Filename	: lc_190920230001.lcb		
Vial #	: 1-2	Sample Type	: Unknown
Injection Volume	: 20 uL		
Date Acquired	: 19-09-2023 10:46:40	Acquired by	: System Administrator
Date Processed	: 19-09-2023 10:54:09	Processed by	: System Administrator

Summary(Compound)
Detector A

1 lc_190920230002.lcd
2 lc_190920230003.lcd
3 lc_190920230004.lcd
4 lc_190920230005.lcd
5 lc_190920230006.lcd

<< Detector A >>
ID#1 Compound Name: Moxonidine

Title	Sample Name	Sample ID	Ret. Time	Area	Height	Conc.
lc_190920230002.lcd	Moxonidine	Std001	2.669	296173	21481	0.000
lc_190920230003.lcd	Moxonidine	Std002	2.669	297009	21379	0.000
lc_190920230004.lcd	Moxonidine	Std003	2.674	296817	21270	0.000
lc_190920230005.lcd	Moxonidine	Std004	2.671	296587	21122	0.000
lc_190920230006.lcd	Moxonidine	Std005	2.672	296869	21041	0.000
Average			2.671	296691	21259	0.000
%RSD			0.072	0.110	0.848	0.000
Maximum			2.674	297009	21481	0.000
Minimum			2.669	296173	21041	0.000
Standard Deviation			0.002	327	180	0.000

Number of Theoretical Plate(USP)	Tailing Factor
987	1.188
971	1.190
1015	1.149
958	1.163
965	1.157
979	1.170
2.303	1.611
1015	1.190
958	1.149
23	0.019

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Sample Information

Sample Name   : Moxonidine (Unsym-0.2)
Sample ID   : BT586
Data Filename   : lc_190920230007.lcd
Method Filename   : Moxonidine Assay.lcm
Batch Filename   : lc_190920230001.lcb
Vial #   : 1-3
Injection Volume : 20 uL
Date Acquired   : 19-09-2023 11:13:57
Date Processed   : 19-09-2023 11:19:05

Sample Type   : Unknown

Acquired by   : System Administrator
Processed by   : System Administrator

Summary(Compound)
Detector A

1 lc_190920230007.lcd
2 lc_190920230008.lcd
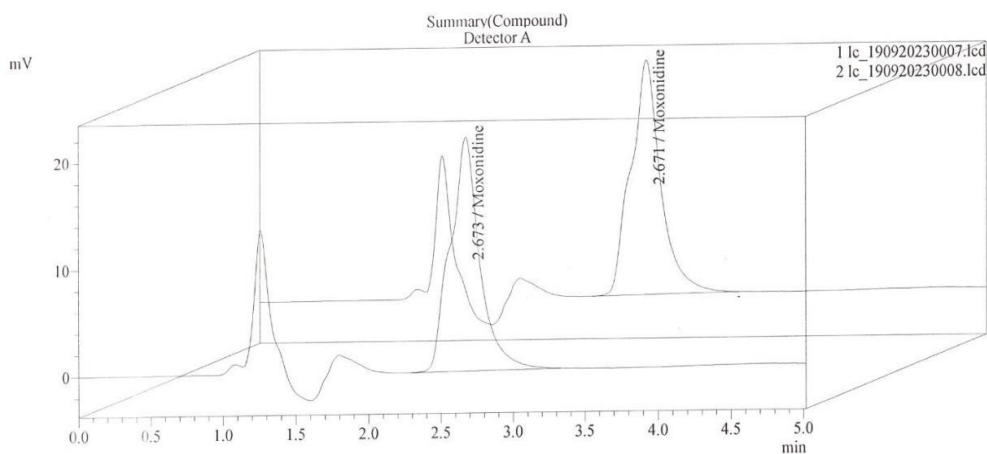
<< Detector A >>
ID#1 Compound Name: Moxonidine

Title	Sample Name	Sample ID	Ret. Time	Area	Height	Conc.
lc_190920230007.lcd	Moxonidine (I	BT586	2.671	309754	21865	0.000
lc_190920230008.lcd	Moxonidine (I	BT586	2.673	310638	21838	0.000
Average			2.672	310196	21851	0.000
%RSD			0.058	0.202	0.086	0.000
Maximum			2.673	310638	21865	0.000
Minimum			2.671	309754	21838	0.000
Standard Deviation			0.002	625	19	0.000

Number of Theoretical Plate(USP)	Tailing Factor
967	1.174
966	1.170
967	1.172
0.076	0.253
967	1.174
966	1.170
1	0.003

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Analytical method validation

HPLC method was validated according to the International Conference on Harmonization guidelines (ICH Q2B, validation of analytical procedures, methodology). The method was validated for parameters such as linearity, precision, accuracy, system suitability limit of detection, limit of quantification and robustness.

This document does not necessarily seek to cover the testing that may be required for registration in, or export to, other areas of the world. The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. A tabular summation of the characteristics applicable to identification, control of assay procedures is included. Other analytical procedures may be considered in future additions to this document.

Linearity

Inject each level (20, 40, 60, 80, 100μg/mL) solutions (prepared from standard stock solution) into HPLC system and observed the linear relationship between concentration and peak area in the concentration range of 20 – 100μg/mL. Calibration curves were plotted with observed peak areas against concentration followed by the determination of regression equations and calculation of the correlation coefficients. 20 μl of each of these working standard solutions of Moxonidine ranging from 1 to 50 μg/ml were injected into a chromatograph at flow rate of 1 ml/min. Retention time and peak area obtained were recorded and standard calibration curve was plotted for Moxonidine, linearity equations were derived. The Correlation coefficient, % curve fitting were also calculated.

Precision

Repeatability

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was calculated.

System precision

Successive 5 injections of 20 μl working standard mixture solution (5 replicates) were injected into a HPLC chromatograph, the peak area and chromatograms obtained were recorded. The % relative standard deviation was calculated for peak areas of replicates.

Method precision

Intra-day Precision

Successive 5 injections of 20 µl of working standard mixture solutions were injected separately at different intervals in the same day and chromatograms were recorded. The % relative standard deviation was calculated for concentration of drug in replicates.

Inter-day Precision

Successive 5 injections of 20 µl of working standard mixture solutions were injected separately on different days and chromatograms were recorded. The % RSD was calculated for concentration of drug in replicates.

Intermediate precision

Intermediate precision (Ruggedness) expresses the variations within laboratories variations: (Different days, different analysts, different equipment, etc.). The Intermediate precision was performed for Moxonidine by different analyst on different instrument using different lot of column on different day.To evaluate the intermediate precision (Also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions. For intermediate precision % RSD was calculated from repeated studies.

Accuracy

20µl solution of the resulting mixture was injected repeatedly into the chromatograph, the peak area and chromatogram obtained were recorded and the % recovery of standard Moxonidine and were calculated. Inject the three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Moxonidine and calculate the individual recovery and mean recovery values.

Limit of Detection and Limit of Quantification

For estimation of LOD and LOQ, visualization method was followed. In visualization method lower dilutions of working standard solution each of Moxonidine of 20µl were injected in to the chromatograph till the drug solution gives response and peak area. The chromatogram and peak area obtained for different concentrations of Moxonidine were recorded.

Specificity

Specificity of a method was determined by testing standard substances against potential interferences. The method was found to be specific when the test solution was injected.

Limit of detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Quantitation limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

Robustness

For the method developed, flow rate of 1 ml/min was used. The robustness study was carried out with small deliberate change to 1 and 1.2ml/min. 20 μl working standard mixture solutions were injected in chromatograph at a flow rate of 1 and 1.2 ml/min, the peak area and chromatograms obtained were recorded. Robustness was done by changing the actual chromatographic conditions like mobile phase ratio and flow rate. Results were determined by calculating the %RSD for injections peak area values of each change in condition.

For the method developed, mobile phase comprising of MEOH: Sodium Dihydrogen Ortho Phosphate (70:30v/v) was used. For Robustness study, the ratio of sodium pantane sulfonate: acetonitrile buffer were slightly altered from the ratio of (834V:136V). 20μl of working standard mixture solutions of Moxonidine were injected in to the chromatograph with altered mobile phase ratios, the peak areas and chromatograms obtained were recorded, and the % assay was calculated.20μl of working standard mixture solutions of Moxonidine were injected in to the chromatograph with altered mobile phase ratios, the peak areas and chromatograms obtained were recorded, and the % assay was calculated.

System suitability

20 μl of standard solutions of Moxonidine were injected into chromatograph and chromatograms were recorded. From the data obtained system suitability parameters like theoretical plates, tailing factor and resolution were calculated.

This parameter used to know whether the HPLC system is suitable for actual chromatographic conditions or not. System suitability was estimated by injecting five standard solutions of Moxonidine and from the chromatograms %RSD, theoretical plates and peak symmetry were calculated.

RESULTS

The objective of the present study was to develop simple and sensitive HPLC method for estimation of Moxonidine. The results obtained for the entire research work are presented here.

Validation parameters

Linearity and Range

The linearity was determined by injecting replicates of working standard solution and found to be in the concentration range of 1-20μg/ml for Moxonidine respectively, with Correlation coefficient, percentage curve fittings found to be well within the acceptance criteria limit. The percentage curve fitting was found to be 99.52% for Moxonidine, respectively.

Precision

Intermediate precision

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. The results were reported in Table1.

Table 1: Results of Intermediate precision for Moxonidine.

S. No	Peak Name	RT	Area (μV*sec)	Height (μV)	USP Plate Count	USP Tailing
1	Moxonidine	5.121	237812	14158	15439	2.306
2	Moxonidine	5.107	541493	13317	15798	2.471
3	Moxonidine	5.109	241979	13256	15465	2.497
4	Moxonidine	5.107	240821	13375	15698	2.492
5	Moxonidine	5.113	238984	13390	15345	2.572
average		5.111	240218	13499	15549	2.468
% RSD		0.111	0.733	2.756	2.629	3.978
Standard deviation		0.006	1761	372	254	0.098

Accuracy

The mean percentage recovery for Moxonidine at three different levels was found to be between 99.00 -105% respectively, which was well within the acceptance limit and hence the

method was found to be accurate. The percentage recovery is within total agreement with acceptance criteria of 90- 110% (table 2).

Table 2: Report of recovery studies for Moxonidine.

Dosage forms	% recovery for moxonidine	Acceptance Criteria
0.2 mg	99.17 %	90- 110%
	104.81 %	90- 110%
	103.38 %	90- 110%
0.3 mg	103.57 %	90- 110%
	101.34 %	90- 110%
	103.30 %	90- 110%

Robustness

The robustness of the method were determined by carrying out the assay after performing slight changes in the mobile phase ratio, detection wavelength and flow rate. It was found that the % assay of Moxonidine ranges between 98% to 105% indicating that the method is robust.

Results suggested that % assay of Moxonidine ranges between 97.5-102% respectively, indicating that the method is robust with respect to slight change in ratio of mobile phase. It was found that % assay of Moxonidine ranges between 99.00-105% respectively, indicating that the method is robust with respect to slight change in detection wavelength (Table No:).

Table 3: Robustness data of Moxonidine with change in flow rate.

Flow rate ml/min		Wavelength in nm	Ratio of mobile phase	peak area* moxonidine	%Assay Moxonidine
0.2 mg	1.2 ml/min	230 nm	864V:136V	494254	104.89 %
	1.2 ml/min	230 nm	864V:136V	400554	103.49%
	1.2 ml/min	230 nm	864V:136V	293713	98.00 %
0.3 mg	1.2 ml/min	230 nm	864V:136V	296842	101.00%
	1.2 ml/min	230 nm	864V:136V	388326	102.00%
	1.2 ml/min	230 nm	864V:136V	266479	102.77%

DISCUSSION

The objective of the present study was to develop simple and sensitive HPLC method for simultaneous estimation of Moxonidine.

A HPLC method was developed with mobile phase consisting of sodium pentane sulfonate & acetonirile 864:136 v/v. The mobile phase was delivered at a flow rate of 1.2 ml/min on C8 column (25 cm X 4mm, 5μm) as stationary phase. Analysis was performed at ambient

temperature with detection at 230nm gave a satisfactory chromatogram of Moxonidine. Different columns and various combinations of organic solvents were tried but, C8 column (25 cm X 4mm, 5µm) column and the mobile phase of Buffer:ACN in ratio 864:136v/vshowed good resolution and was used. Standard solutions of Moxonidine (20µg/ml) were injected into chromatograph and scanned in the wavelength range of 200-400 nm, the overlain UV spectrum of Moxonidine was prepared and isobestic point was found to be at 230 nm. The retention time of Moxonidine were found to be aprox at 2-6 min, respectively.

The developed method was then validated by using various parameters like Linearity, Precision, Accuracy, Robustness and Ruggedness etc. as per ICH guidelines.

The linearity was determined by injecting replicates of working standard solution and found to be in the concentration range of 1-20µg/ml for Moxonidine respectively. The linearity graph for the drugs is satisfactory as observed from the correlation coefficient (R^2) values of for Moxonidine respectively.

The method is specific for estimation of Moxonidine as no other peak could be detected in the retention time upto 15 min with diluent.

As all the values of % RSD for precision study obtained are within the acceptance criteria of less than 2%, the proposed method is found to show good degree of precision and reproducibility. In determination of accuracy, the percentage recovery is with in total agreement with acceptance criteria of 90- 110%.

The robustness of the method were determined by carrying out the assay after performing slight changes in the mobile phase ratio, detection wavelength and flow rate. All the robustness results indicated that the new method developed was robust and did not show significant variations on slight changes in the mobile phase ratio, detection wavelength and flow rate.

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