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METHOD DEVELOPMENT AND VALIDATION OF PHENTERMINE BY USING UPLC METHOD

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ABSTRACT

A simple and selective UPLC method is described for the determination of Phentermine. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of 40 volumes of Methanol, 40 volumes of Acetonitrile and 20 volumes of Water with detection of 263 nm. Linearity was observed in the range 50-150 µg /ml for Phentermine (r^2 =0.990) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Key Words: Phentermine, UPLC method

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INTRODUCTION

The number of drugs introduced into the market is increasing every year. These drugs may be either new entities or partial structural modification of the existing one. Often a time lag exists from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, reports of new toxicities (Resulting in their withdrawal from the market), development of patient resistance and introduction of better drugs by competitors. Under these conditions, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary, therefore to develop newer analytical methods for such drugs. Analytical methods should be used within good manufacturing practice (GMP) and good laboratory practice (GLP) environments, and must be developed using the protocols set out in the International Conference on Harmonization (ICH) guidelines (Q2A and Q2B). Method development is a continuous process that progresses in parallel with the evolution of the drug product.

The goal and purpose of the method should reflect the phase of drug development. During early drug development, the methods may focus on API behavior. They should be suitable to support preclinical safety evaluations, pre-formulation studies, and prototype product stability studies. As drug development progresses, the analytical methods are refined and expanded, based on increased API and drug product knowledge. The methods should be robust and uncomplicated, while still meeting the appropriate regulatory guidelines. Scouting experiments are frequently performed during method development to establish the performance limits of the method, prior to formal validation experiments. These may include forced degradation studies, which are an integral part of development of a stability-indicating method. API is typically subjected to degradation by acid, base, peroxide, heat, and light. This allows for a determination of the capability of the method to separate and quantify degradation products, while providing insight into the main mechanisms of degradation. Once a stability-indicating method is in place, the formulated drug product can then be subjected to heat and light in order to evaluate potential degradation of the API in the presence of formulation excipients.

In method development, an attempt to select the best chromatographic conditions like the best column, the best mobile phase, the detection wavelength etc. to be used for routine analysis of any drug is done. For the method development by UPLC method some information about the sample is very essential i.e. number of components present in the sample, pKa values of different components, UV-Visible Spectra of each analyte, solubility in different solvents, concentration range of each component, nature of sample etc. Prior to method development there must be some technical information i.e. chromatography method selection according to the sample properties, the sample when analyzed with UPLC, the condition where all compounds elute in a reasonable time, optimization of UPLC method with regard to analysis time, resolution, selectivity and sensitivity (1-6).

Phentermine also known as α,α-dimethylphenethylamine, is a psychostimulant drug of the substituted amphetamine chemical class, with pharmacology similar to amphetamine. It is used medically as an appetite suppressant for short term use, as an adjunct to exercise and reducing calorie intake. Phentermine is an amphetamine that stimulates neurons to release or maintain high levels of a particular group of neurotransmitters known as catecholamines; these include dopamine and norepinephrine. High levels of these catecholamines tend to suppress hunger signals and appetite. The drug seems to inhibit reuptake of noradrenaline, dopamine, and seratonin through inhibition or reversal of the reuptake transporters. It may also inhibit MAO enzymes leaving more neurotransmitter available at the synapse. Phentermine (through catecholamine elevation) may also indirectly affect leptin levels in the brain. It is theorized that phentermine can raise levels of leptin which signal satiety. It is also theorized that increased levels of the catecholamines are partially responsible for halting another chemical messenger known as neuropeptide Y. This peptide initiates eating, decreases energy expenditure, and increases fat storage. Opioids close N-type voltage-operated calcium channels (kappa-receptor agonist) and open calcium-dependent inwardly rectifying potassium channels (mu and delta receptor agonist). This results in hyperpolarization and reduced neuronal excitability. Phentermine is indicated in the management of exogenous obesity as a short term (a few weeks) adjunct in a regimen of weight reduction based on caloric restriction. Phentermine hydrochloride is a sympathomimetic amine with pharmacologic activity similar to the prototype drugs of this class used in obesity, the amphetamines. Actions include central nervous system stimulation and elevation of blood pressure (7).Aim is to develop and validate new UPLC method for Phentermine in pharmaceutical dosage form.

MATERIALS AND METHODS

Determination of Working Wavelength (λmax) (8-10)

Preparation of standard stock solution of Phentermine

10 mg of Phentermine was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Assay

Preparation of samples for Assay

Preparation of mixed standard solution

Weigh accurately 10 mg of Phentermine in 25 ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 20 µg/ml of Phentermine is prepared by diluting 0.5ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Sample preparation

Weigh accurately 10 Tablets **(Adipex - P 37.5 mg)** weigh accurately 10 mg of Phentermine in 25 ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 20 µg/ml of Phentermine is prepared by diluting 0.5ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Calculation

The amount of Phentermine present in the formulation by using the formula given below, and results shown in above table:

$$\%\ \text{Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

Validation

To verify that the analytical system is working properly and can give accurate and precise results were evaluated by 20µg/mL of phentermine was injected six times and the chromatograms were recorded for the same.

RESULTS AND DISCUSSION

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drug in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The absorption curve shows characteristic absorption maxima at 263nm for Phentermine, selected as detector wavelength for the HPLC chromatographic method.

The amount of Phentermine present in the taken dosage form was found to be 100.90% respectively (Fig-1-5).

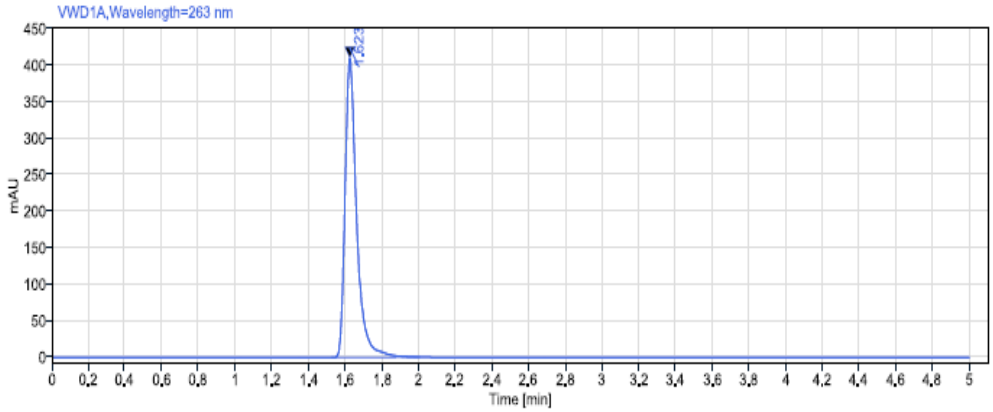

Fig-1 Chromatogram of Assay sample preparation-1

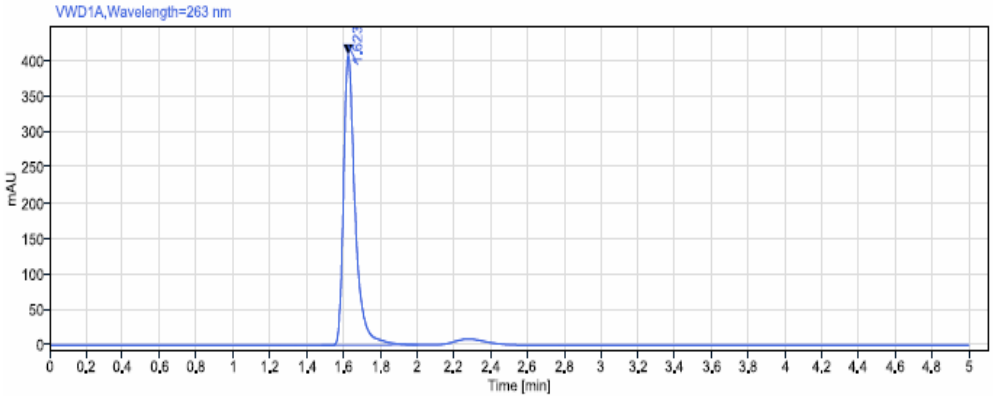

Fig-2 Chromatogram of Assay sample preparation-2

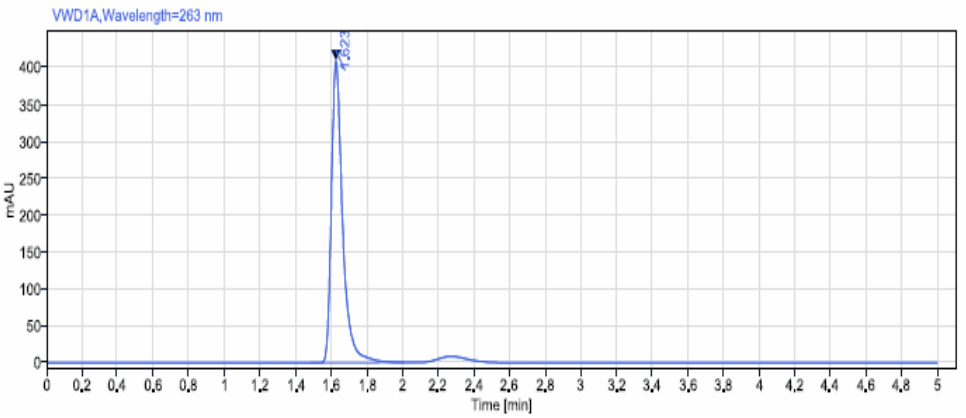

Fig-3 Chromatogram of Assay sample preparation-3

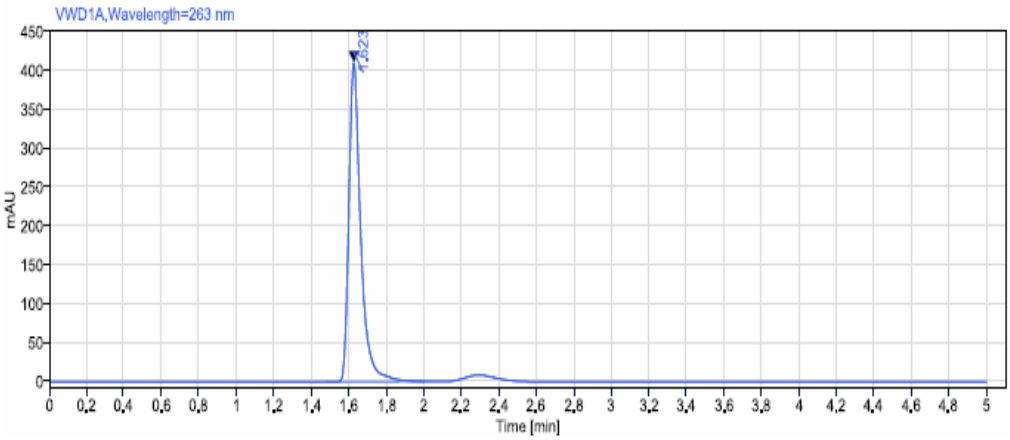

Fig-4 Chromatogram of Assay sample preparation-4

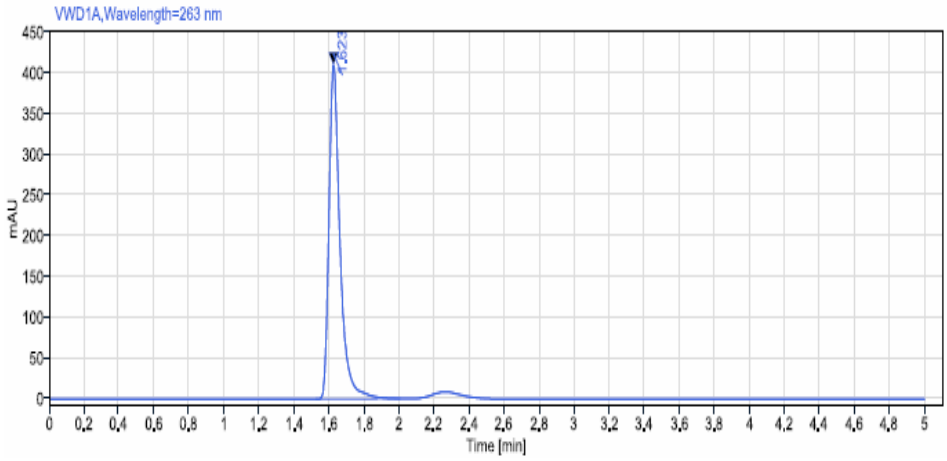

Fig-5 Chromatogram of Assay sample preparation-5

The plate count and tailing factor results were found to be within the limits and the % RSD was found to be 1.2 so system is suitable and giving precise results (Table-1).

Table-1 Results for system suitability of phentermine

Injection	RT	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	1.622	1806.55	3700	1.53
2	1.623	1807.57	3686	1.48
3	1.623	1808.12	3682	1.48
4	1.623	1808.46	3682	1.48
5	1.623	1809.14	3682	1.48
6	1.623	1809.18	3685	1.47
Mean	1.623	1808.170	-	-
SD	0.00041	1.00	-	-
%RSD	0.025	0.06	-	-

The %RSD of Assay for 6 Samples determinations of phentermine found to be within the acceptance criteria (less than 2.0%). hence method is precise (Table-2).

Table-2 Method precision results for PHENTERMINE

Phentermine		
S.No.	RT	AREA
1	1.623	1792.8
2	1.624	1793.47
3	1.624	1795.84
4	1.624	1797.36
5	1.624	1798.51
6	1.624	1800.08
AVG	1.6238	1796.3433
SD	0.0004	2.86
%RSD	0.025	0.16

The correlation coefficient for linear curve obtained between concentrations vs. Area for standard preparation 0.990. The Average % recovery of phentermine between 98% and 102%. From the above results % Assay and %RSD obtained acceptance criteria so method is rugged.

CONCLUSION

The above experimental results and parameters it was concluded that, this newly developed method for the estimation of phentermine was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

REFERENCES

1. Chatwal**,** R**.** G**.;** Anand**,** K**.** S**.** *High performance liquid chromatography***.** *Instrumental methods of chemical analysis***,** 5thed**.;** Himalaya publishers**:** Mumbai, 2010**;** 2.570-2.629**.**
2. Sharma**,** B**.** K**.** *High performance liquid chromatography***.** *Instrumental methods of chemical analysis***,** 24th ed**.;** Goelpublishers**:** Meerut**,** 2005**;** 295 - 300**.**
3. Dong,W. M. *HPLC instrumentation and trends.* Modern HPLC for practicing scientists, USA, 2006; 5-10, 78-110.
4. *Typical diagram of UPLC* http://www.comsol.com/stories/waters_corp_hplc_systems/full/ Hplc diagram
5. *UHPLC solvent properties* www.sanderkok.com/techniques/hplc/eluotropic_series_extended.htm
6. Swartz, M. E.; Ira Krull, S, *Analytical method development. Analytical method development and validation*, 1st ed.; Marcel Dekker, Inc: New York, 2009; 17-80.
7. https://www.drugbank.ca/categories/DBCAT001351
8. ICH, *Text on Validation of Analytical Procedures,* ICH – Q2A, International Conference on Harmonisation, IFPMA, Geneva, 1995, 2-3, A–1 to A–3.
9. ICH, *Validation of Analytical Procedures: Methodology*, ICH – Q2B, International Conference on Harmonisation, 1996, 1-3.
10. ICH *Guidelines, Q2 (R1) - Validation of Analytical Procedures*: Text and Methodology,2005, 1-6.