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Development and Validation of RP-HPLC Method for the determination of Methylphenidate Hydrochloride in API

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Abstract: A linear, precise, accurate and robustic RP-HPLC method has been developed and validated for the quantitative determination of methylphenidate hydrochloride in active pharmaceutical ingredient. Chromatography was carried out on enable C_{18} G column (250×4.6mm, 5µ) with a mobile phase of acetonitrile-methanol-0.1% formic acid in the ratio of 45:45:10 (v/v/v). UV detection was performed at 220 nm. Linearity was observed in the concentration range of 20-45 µg/ml with a correlation coefficient (r^2) of 0.9981. The retention time for methylphenidate was found to be 5.7 min. The proposed method can be successfully applied for the estimation of methylphenidate in bulk. Methylphenidate hydrochloride was subjected to stress conditions including acidic, alkaline, and thermal degradation. Methylphenidate hydrochloride is more sensitive to wards acidic and alkaline degradation. The method was validated as per ICH guidelines.
Key words: Methylphenidate hydrochloride, RP-HPLC, Validation, Method development.

Introduction

Methylphenidate is a psycho-stimulant drug approved for the treatment of attention deficit hypersensitivity disorder (ADHD), postural orthostatic tachycardia syndrome and narcolepsy. Methylphenidate is the most commonly prescribed psycho-stimulant and works by increasing the activity of central nervous system.[1] Chemically, methylphenidate is methyl-2-phenyl-2-(piperidin-2-yl)acetate (Fig 1). It blocks dopamine uptake in central adrenergic neurons by blocking dopamine transport or carrier proteins. Methylphenidate acts at the brain stem arousal system and the cerebral cortex and causes increased sympathomimetic activity in the central nervous system.[2]

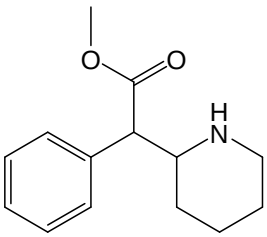

Fig 1: Chemical structure of Methylphenidate

Some researchers have theorized that ADHD is caused by a dopamine imbalance in the brains of those affected. Methylphenidate is a norepinephrine and dopamine reuptake inhibitor, which means that it increases the level of the dopamine neurotransmitter in the brain by partially blocking the dopamine transporter (DAT) that removes dopamine from the synapses. This inhibition of DAT blocks the reuptake of dopamine and norepinephrine into the presynaptic neuron, increasing the amount of dopamine in the synapse.[2,3]

Literature survey revealed that only few analytical methods like spectrophotometric, RP-HPLC and LC-MS methods[4-8] have been reported for the determination of methylphenidate. All these methods are expensive, time consuming, complex in nature. Consequently, there was still a need to develop a simple, less time consuming and economical method for the determination of Methylphenidate in API Methylphenidate. Therefore, we attempted to develop a fast and reproducible RP-HPLC method for the estimation Methylphenidate in API form by following ICH method validation guidelines.[9] In the present work, we developed a simple, precise, accurate, selective and robust liquid chromatographic method for the determination of Methylphenidate in active pharmaceutical ingredient as an alternative method.

Materials and Methods:

Methylphenidate was obtained as gift sample from ZCL Chemicals Ltd. Ankleshwar, Gujarat. All the solvents used like methanol, acetonitrile and formic acid which are of HPLC grade were purchased from SD Fine Chemicals.

Instrumentation and analytical conditions

The analysis of the drug was carried out on Schimadzu HPLC model with LC-10 software containing LC-20AT pump, UV/Visible detector (SPD 20 A), and hamilton syringe (20 µl). Chromatographic analysis was performed by using enable C_{18} G column (250×4.6mm, 5µ). Schimadzu electronic balance was used for weighing. Isocratic elution was performed by using a mobile phase acetonitrile : methanol : 0.1% formic acid (45:45:10) at a flow rate of 1.0 ml/min. Detection was carried out at 220 nm with a run time of 20 min. The mobile phase was prepared freshly and it was sonicated to degas the solvent for 5 min. The column and HPLC system were maintained at ambient temperature.

Preparation of stock, working standard and sample solutions

An accurate quantity of powder equivalent to 100 mg of methylphenidate was weighed and transferred to a 100 ml volumetric flask containing methanol and diluted up to the mark with methanol, shaken for 5 min, sonicated for 15 min and filtered through 0.45 µ membrane filter to obtain a clear solution. From the primary stock solution, 10 ml was taken in a 100 ml volumetric flask and dilute up to the mark with methanol and sonicated. This secondary stock sample solution was diluted quantitatively with methanol to obtain suitable working sample solutions for chromatographic measurements.

Method Validation

The proposed chromatographic method was validated as per ICH guidelines.[9] Peak calibration curve was constructed by plotting peak area Vs concentration. Accuracy was determined by recovery studies with known concentration of drugs and the percentage recoveries of the added drugs were determined. Precision was evaluated in terms of intra-day and inter-day precision. The precision was investigated using six replicates of same concentrations of standard solutions. LOD and LOQ values were calculated from the calibration curve. Robustness of the method was determined by deliberately varying certain parameters like flow-rate, analytical wavelength and column temperature.

Forced Degradation Studies:

The study was intended to ensure the effective separation of methylphenidate and its degradation peaks of formulation ingredients at the retention time of methylphenidate. Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method.[10] Methylphenidate standard solution of concentration 1000 µg/ml was prepared with mobile phase and treated with 5 ml of 1N HCl. The resultant solution was analysed for every 24 h after prior dilution. For alkaline degradation study methylphenidate standard solution of concentration 1000 µg/ml was prepared with mobile phase and treated with 5 ml of 1N NaOH. The resultant solution was analysed for every 24 h after prior dilution. Methylphenidate powder was exposed to dry heat at 60^0 C and powder was removed for every 24 h and diluted as mentioned above and analysed for thermal degradation study.

Results and discussion

Method development:

Proper selection of the method depends upon the nature of the sample (ionic/ionisable/neutral molecule), its molecular weight and solubility. The drug selected in the present study is polar in nature. The reversed phase HPLC was selected for the separation because of its simplicity and suitability. The sensitivity of HPLC method which uses UV detection depends upon the proper selection of wavelength. An ideal wavelength is one that gives good response for all the drugs to be detected. A UV spectrum for methylphenidate was recorded between 200-400 nm. The λ_{max} was obtained at 220 nm by using 1cm quartz cell. Different mobile phases were tried but satisfactory separation and symmetrical peaks were obtained by using a mobile phase consisting of acetonitrile : methanol : 0.1% formic acid in the ratio 45:45:10.

Linearity:

Appropriate aliquots of standard methylphenidate solution were taken and diluted up to the mark with HPLC grade methanol to obtain final concentrations ranging from 20-45 µg/ml. These solutions were injected into chromatographic system. The chromatograms were obtained and peak area was determined for each concentration of drug solution. The correlation coefficient was found to be 0.9981. Results of linearity are shown in Table 1, Fig 2.

Table 1. Linearity data of methylphenidate hydrochloride

S.No	Concentration(µg/ml)	Peak area
1	20	858200
2	25	1154211
3	30	1387440
4	35	1668846
5	40	2003083
6	45	2256898

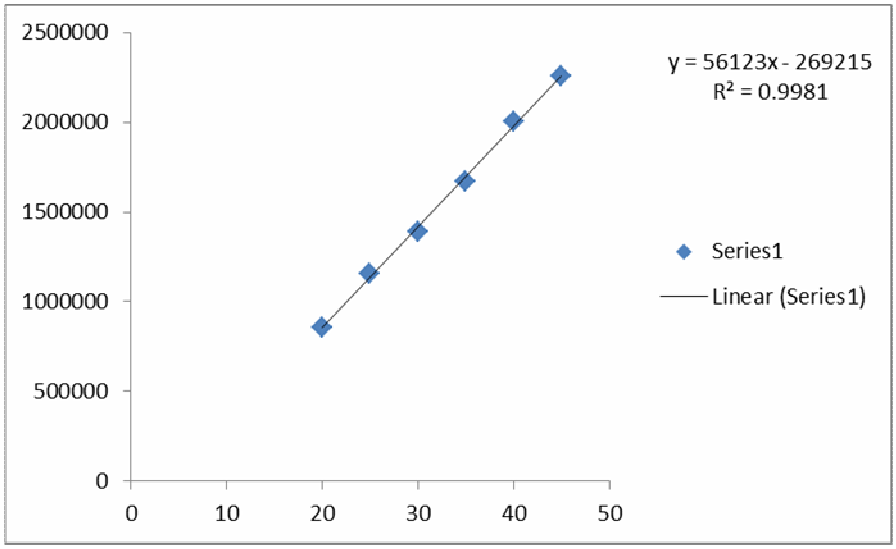

Fig.2 Calibration curve for methylphenidate hydrochloride (20-45 µg/ml)

Accuracy

Accuracy of the method was determined by recovery experiments. Two types of recovery studies were performed. They are standard addition and percentage recovery methods. The amount of the each drug present, percentage recovery, percentage relative standard deviation (% RSD) were calculated. The percentage recovery was found to be 99.13%-99.76%.

Precision

Precision of the method was demonstrated by inter day and intraday variation studies. It was checked by injecting replicate injections of 25 µg/ml of the solution for three times in a day as intraday precision study of methylphenidate and for three consecutive days as interday precision. % RSD was calculated. The % RSD obtained for intraday and interday precision was less than 0.9% as shown in Table 2.

Sensitivity

The sensitivity of measurement of methylphenidate by use of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ).

The LOD and LOQ were calculated by use of the equations:

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Limit of Detection (LOD) and Limit of Quantitation (LOQ) were estimated from signal-to-noise ratio. Limit of Detection is defined as the lowest concentration level resulting in a peak height of three times the baseline noise. Limit of Quantitation is defined as the lowest concentration level that provided a peak height with a signal-to-noise ratio higher than 10. The LOD and LOQ values for methylphenidate are reported in the Table 3.

Table 2. Intra and inter day precision of Methylphenidate

Sample	Concentration (µg/ml)	%RSD	
		Intra	Inter
Methylphenidate hydrochloride	20	0.7	0.4
	25	0.6	0.7
	30	0.7	0.4
	35	0.9	0.7
	40	0.2	0.6
	45	0.6	0.9

Table.3 LOD and LOQ results of methylphenidate hydrochloride

Sample	LOD (µg/ml)	LOQ (µg/ml)
Methylphenidate hydrochloride	1.50	4.54

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. For the determination of a method's robustness, deliberate change in the flow rate, wavelength and temperature variation was made to evaluate the impact on the method. Results of robustness are summarized in Table 4. The method was found to remain unaffected by changing the method parameters. Hence, the method was found to be robust.

Table 4: Robustness results for methylphenidate hydrochloride

Factor		%RSD
Flow rate (ml/min)	0.8	0.56
	1	0.67
	1.2	0.42
Wavelength (nm)	218	0.61
	220	0.83
	222	1.20
Column temperature (^{0} C)	28	0.87
	30	0.83
	32	0.61

Forced degradation studies:

All the stressed samples in acid, alkaline degradation studies were decomposed completely. No decomposition was seen on exposure of solid drug to dry heat. The forced degradation studies data are summarized in Table 5.

Table 5. Data of forced degradation studies

S:NO	Stress condition	Time	Degradation (%)
1	Acid hydrolysis (1N HCl)	24 h	100
2	Alkaline hydrolysis (1N NaOH)	48 h	100
4	Thermal degradation (60^0C)	7 days	Stable

Conclusion

A simple, sensitive, specific, accurate and precise stability indicating RP-HPLC method was developed and validated for the routine analysis methylphenidate in API. The % RSD for all parameters were found to be less than two, which revealed the validation of new method are fairly satisfactory. The results of forced degradation studies reveal that the method is stability indicating. The proposed method has the capability to separate the analyte from their degradation products obtained during forced degradation studies. Hence, it can be concluded that the developed RP-HPLC method is stability indicating and can be employed successfully as an alternative for HPLC and LC-MS methods for the quantitative estimation of methylphenidate.

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