Research Article

Journal of Atoms and Molecules
An International Online Journal
ISSN – 2277 – 1247

RP – HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ANALYSIS OF METHYL PHENIDETE HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORMS.

Reshma Syed[1], Kalpana Pammi [1*], B.Sandhya [2]
[1]Department of Biochemistry, Acharya Nagarjuna University, Guntur, Andhrapradesh.
[2]Dept of Life Sciences, SIMS College, Guntur, A.P, India.

Received on: 05-11-2011 Revised on: 10-12-2011 Accepted on: 24–12–2011

Abstract:

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Methyl phenidete hydrochloride in tablet dosage form. Isocratic elution at a flow rate of 1ml min^{-1} was employed on a symmetry C18 column at ambient temperature. The mobile phase consisted of Methanol:acetonitrile:THF 75:20:05 (v/v/v). The UV detection wavelength was at 278nm.Linearity was observed in concentration range of 20-55ppm. The retention time for Methyl phenidete hydrochloride was 3.32 min. The method was validated as per the ICH guidelines. The proposed method can be successfully applied for the estimation of Methyl phenidete hydrochloride in pharmaceutical dosage forms.

Key Words:

Methyl phenidete hydrochloride, HPLC, Linearity, Precision, 278nm

* Corresponding author

Kalpana Pammi,
Email: kalpana.pammi@gmail.com
Tel: +

Introduction:

Methylphenidate is psychostimulant drug appr oved for treatment of attention-deficit hyperactivity disorder, postural orthostatic tachycardia syndrome and narcolepsy. It may also be prescribed for off-label use in treatment-resistant cases of lethargy, depression, neural insult and obesity. Methylphenidate belongs to the piperidine class of compounds and increases the levels of dopamine and norepinephrine in the brain

through reuptake inhibition of the monoamine transporters. Methylphenidate possesses structural similarities to amphetamine and its pharmacological effects are more similar to those of cocaine, though MPH is less potent and longer in duration of action.[1,2,3.]

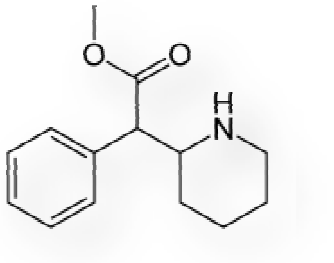


Figure 1: Stricture of Methylphenidate

MPH is the most commonly prescribed psychostimulant and works by increasing the activity of the central nervous system.[4] It produces such effects as increasing or maintaining alertness, combating fatigue, and improving attention.[5] The short-term benefits and cost effectiveness of methylphenidate are well established, although long-term effects are unknown.[6,7] The long term effects of methylphenidate on the developing brain are unknown. Methylphenidate is not approved for children under six years of age.[8,9] Methylphenidate is approved by the U.S. Food and Drug Administration (FDA) for the treatment of attention-deficit hyperactivity disorder[10] The addition of behavioural modification therapy (e.g. cognitive behavioral therapy (CBT)) has additional benefits on treatment outcome.[11,12] There is a lack of evidence of the effectiveness in the long term of beneficial effects of methylphenidate with regard to learning and academic performance.[13] One study found that pharmacological treatment of ADHD in childhood reduces the risk that children will resort to substance abuse in adolescence by 85%, while untreated ADHD was a significant risk factor in developing substance abuse.[14] A meta analysis of the literature concluded that methylphenidate quickly and effectively reduces the signs and symptoms of ADHD in children under the age of 18 in the short term but found that this conclusion may be biased due to the high number of low quality clinical trials in the literature. There have been no placebo controlled trials investigating the long term effectiveness of methylphenidate beyond 4 weeks thus the long term effectiveness of methylphenidate has not been scientifically demonstrated. Serious concerns of publication bias regarding the use of methylphenidate for ADHD have also been noted.[15] A diagnosis of ADHD must be confirmed and the benefits and risks and proper use of stimulants as well as alternative treatments should be discussed with the parent before stimulants are prescribed.[16] The dosage used can vary quite significantly from individual child to individual child with some children responding to quite low doses whereas other children require the higher dose range. The dose, therefore, should be titrated to an optimal level that achieves therapeutic

benefit and minimal side-effects.[17] This can range from anywhere between 5–30 mg twice daily or up to 60 mg a day. Therapy with methylphenidate should not be indefinite. Weaning off periods to assess symptoms are recommended.[18]

Use of stimulants such as methylphenidate in cases of treatment resistant depression is controversial. In individuals with cancer, methylphenidate is commonly used to counteract opioid-induced somnolence, to increase the analgesic effects of opioids, to treat depression, and to improve cognitive function.Methylphenidate may be used in addition to an antidepressant for treatment-refractory major depressive disorder. It can also improve depression in several groups including stroke, cancer, and HIV-positive patients. However, benefits tend to be only partial with stimulants being, in general, less effective than traditional anti-depressants and there is some suggestive evidence of a risk of habituation. Stimulants may however, have fewer side-effects than tricyclic antidepressants in the elderly and medically ill. A review of the literature found that methylphenidate was ineffective for refractory cases of major depression.

Methylphenidate has shown some benefits as a replacement therapy for individuals dependent on methamphetamine. Cocaine and methamphetamine interfere with the protein DAT, over time causing DAT upregulation and lower cytoplasmic dopamine levels in their absence. Methylphenidate and amphetamine have been investigated as a chemical replacement for the treatment of cocaine dependence in the same way that methadone is used as a replacement for heroin. Its effectiveness in treatment of cocaine or other psychostimulant dependence has not been proven and further research is needed.

Early research began in 2007–2008 n some countries on the effectiveness of methylphenidate as a substitute agent in refractory cases of cocaine dependence, owing to methylphenidate's longer half life, and reduced vasoconstrictive effects. This replacement therapy is used in other classes of drugs such as opiates for maintenance and gradual withdrawal such as methadone, suboxone, etc. Animal studies using rats with ADHD-like behaviors were used to assess the safety of methylphenidate on the developing brain and found that psychomotor impairments, structural and functional parameters of the dopaminergic system were improved with treatment. This animal data suggests that methylphenidate supports brain development and hyperactivity in children diagnosed with ADHD. However, in normal control animals methylphenidate caused long lasting changes to the dopaminergic system suggesting that if a child is misdiagnosed with ADHD they may be at risk of long lasting adverse effects to brain development. Animal tests found that rats given methylphenidate

grew up to be more stressed and emotional. It is unclear due to lack of follow up study whether this occurs in ADHD like animals and whether it occurs in humans. However, long lasting benefits of stimulant drugs have not been found in humans. Narcolepsy, a chronic sleep disorder characterized by overwhelming daytime drowsiness and sudden need for sleep, is treated primarily with stimulants. Methylphenidate is considered effective in increasing wakefulness, vigilance, and performance. Methylphenidate improves measures of somnolence on standardized tests, such as the Multiple Sleep Latency Test, but performance does not improve to levels comparable to healthy controls.

Experimental

Chemicals and reagents

All HPLC solvents used like Acetonitrile, Methanol,Orthophosphoric Acid which are of HPLC grade were purchased from E.Merck,

Instrumentation and analytical conditions

The analysis of the drug was carried out on Shimadzu HPLC model (VP series) containing LC-10AT (VP series) pump, variable wave length programmable UV/visible detector SPD-10AVP and rheodyne injector (7725i) with 20µl fixed loop. Chromatographic analysis was performed using Inertsil ODS C-18 column with 250 x 4.6mm internal diameter and5µm particle size. Shimadzu electronic balance (AX-200) was used for weighing. Isocratic elution with Methanol,Acetonitrile:THF 75:20:05 (v/v/v) was selected with a flow rate of 1.0 ml min^{-1}.The detection wavelength was set at 278nm with a runtime of 10 min. The mobile phase was prepared freshly and it was degassed by sonicating for 5 min before use. The column was equilibrated for at least 30min with the mobile phase flowing through the system. The column and the HPLC system were kept at ambient temperature.

Preparation of Stock, working standard solutions and Sample solutions

100mg of Methyl phenidete hydrochloride was weighed and transferred (working standard) into a 100ml volumetric flask. The diluent methanol was added and sonicated to dissolve it completely and made up to the mark with the same solvent. Further 1ml of the above stock solution was pipetted into a 10ml volumetric flask and diluted up to the mark with diluent. The contents were mixed well and filtered through Ultipor N_{66} Nylon 6, 6 membrane sample filter paper. The calibration curve was plotted with the concentrations of the 20 to 55ppm working standard solutions. Calibration solutions were prepared and analyzed immediately after preparation.

The formulation tablets of Methyl phenidete hydrochloride were crushed to give finely powdered material. Powder equivalent to 25 mg of drug was taken in 10 ml of volumetric

flask containing 5 ml of mobile phase and was shaken to dissolve the drug and then filtered through Ultipor N_{66} Nylon 6,6 membrane sample filter paper. Volume of the filtrate was adjusted to the mark with the same solvent to obtain 40 ppm concentration.

Method Validation procedure

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision, accuracy, specificity, and limit of detection, limit of quantification, robustness and system suitability.

Linearity

The developed method has been validated as per ICH guidelines (Zucman D, 2007). Working standard solutions of Methyl phenidete hydrochloride in the mass concentration range of 20 to 55ppm was injected into the chromatographic system. The chromatograms were developed and the peak area was determined for each concentration of the drug solution. Calibration curve of Methyl phenidete hydrochloride was obtained by plotting the peak area ratio versus the applied concentrations of Methyl phenidete hydrochloride. The linear correlation coefficient was found to be 0.999.

S.NO	CONC (ppm)	AREA
1	20	15972
2	25	18973
3	30	22351
4	35	26784
5	40	31298
6	45	33472
7	50	37072
8	55	41486

Table 1: Linearity of Methyl phenidete hydrochloride

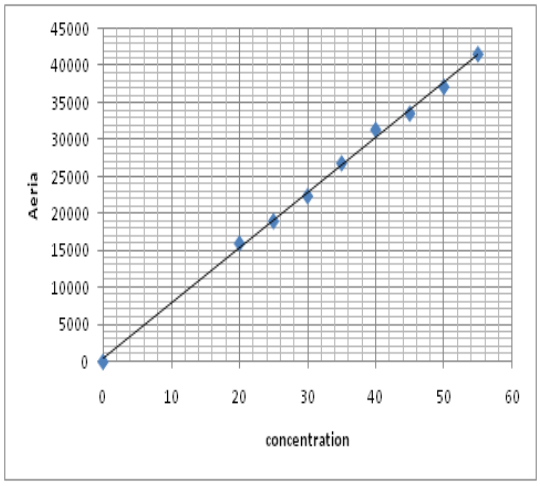


Figure 2: Calibration curve of Methyl phenidete hydrochloride

Drug	Methyl phenidete hydrochloride
Concentration range	20-55ppm
Slope (m)	1158.721
Intercept (b)	413.087
Correlation coefficient	0.999
% RSD	0.28

Table.2: Linear Regression Data for Calibration curve

Precision

Repeatability of the method was checked by injecting replicate injections of 40 ppm of the solution for six times on the same day as intraday precision study of Methyl phenidete hydrochloride and the RSD was found to be 0.28.

INJECTION	CONCENTRATION	INTERDAY	INTRADAY
1	40ppm	29967	29825
2	40ppm	29875	29951
3	40ppm	29998	29762
4	40ppm	30075	29775
5	40ppm	29851	29889
6	40ppml	29882	30019
	RSD	0.30	0.26

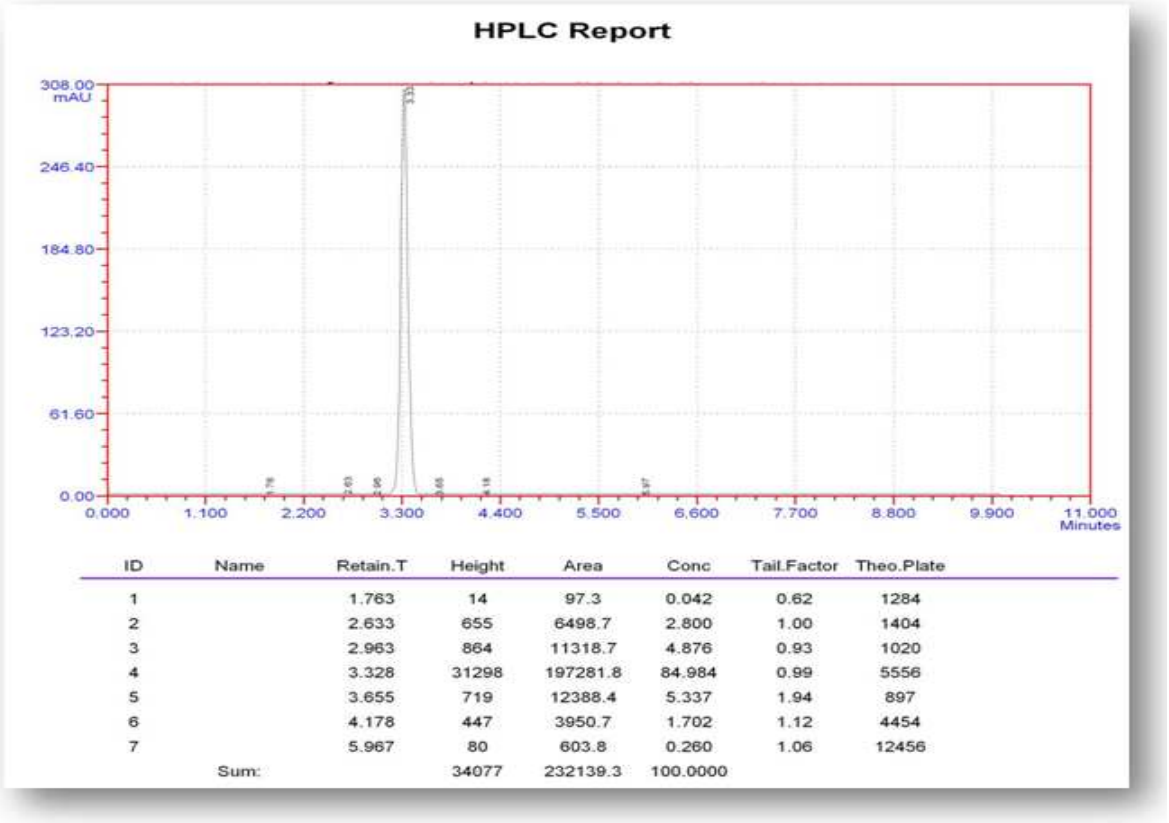
Table 3: Precision parameters of Methyl phenidete hydrochloride

Accuracy

The accuracy of the method was determined by calculating recovery of Methyl phenidete hydrochloride (0.2, 0.4, 0.6 mg/ml) by the method of standard addition. Known amount of Methyl phenidete hydrochloride was added to a pre quantified sample solution and the amount of Methyl phenidete hydrochloride was estimated by measuring the peak area ratios and by fitting these values to the straight line equation of calibration curve. The recovery studies were carried out three times over the specified concentration range and amount of Methyl phenidete hydrochloride was estimated by measuring the peak area ratios by fitting these values to the straight line equation of calibration curve. From the above determination, percentage recovery and standard deviation of percentage recovery were calculated.

Recovey	Conc. of sample	Recovery	% of recovery
50%	15ppm	14.97	99.8
100%	30ppm	29.88	99.60
150 %	45ppm	45.09	100.2

Table 4: Accuracy results of Methyl phenidete hydrochloride



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1		1.763	14	97.3	0.042	0.62	1284
2		2.633	655	6498.7	2.800	1.00	1404
3		2.963	864	11318.7	4.876	0.93	1020
4		3.328	31298	197281.8	84.984	0.99	5556
5		3.655	719	12388.4	5.337	1.94	897
6		4.178	447	3950.7	1.702	1.12	4454
7		5.967	80	603.8	0.260	1.06	12456
	Sum:		34077	232139.3	100.0000		

Figure 3: Typical chromatogram of Methyl phenidete hydrochloride

Specificity

The specificity of the method was determined by comparing test results obtained from analysis of sample solution containing excipients with that of test results those obtained from standard drug.

LOD and LOQ

Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 50microgram/ml and 10microliter respectively as per ICH guide-lines.

Parameter	Measured
LOD	0.05ppm
LOQ	0.15ppm

Table 5 : LOD and LOQ Results

Ruggedness

Inter day variations were performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different days over a period of one week. Ruggedness also expressed in terms of percentage relative standard deviation.

Robustness

To determine the robustness of the method, two parameters from the optimized chromatographic conditions were varied.

Parameter	Modification	Peak Area	% of change
M.PHASE	Methanlo: acetonitrile: THF 70:25:5	29891	0.99
PH	5.5	30152	1.06
WAVELENGTH	285	29972	1.00

Table 6 : Robustness Results.

System Suitability Parameter:

System suitability tests were carried out on freshly prepared standard stock solutions of Methyl phenidete hydrochloride and it was calculated by determining the standard deviation of Methyl phenidete hydrochloride standards by injecting standards in six replicates at 6 minutes interval and the values were recorded

Parameters	Values
λ max (nm)	278
Beer's law limit (µg/ml)	20-55ppm
Correlation coefficient	0.999
Retention time	3.3min
Theoretical plates	5556
Tailing factor	0.99
Limit of detection	0.05ppm
Limit of quantification	0.15ppm

Table 7 : System Suitab ility Parameter

Result and discussion:

Optimization of the chromatographic conditions

The nature of the sample, its molecular weight and solubility decides the proper selection of the stationary phase. The drug Methyl phenidete hydrochloride being non-polar is preferably analyzed by reverse phase columns and accordingly C18 column was selected. So the elution of the compound from the column was influenced by polar mobile phase. The concentration of the methanol and Acetonitrile were optimized to give symmetric peak with short run time based on asymmetric factor and peak area obtained. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase Methanol:acetonitrile:THF 75:20:05 (V/V/V). The retention time of Methyl phenidete hydrochloride was found to be 3.3min , which indicates a good base line. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise. The system suitability and validation parameters are given in Table 4. The high percentage of recovery of Methyl phenidete hydrochloride was found to be 99.28 indicating that the proposed method is highly accurate. Proposed liquid chromatographic method was applied for the determination of Methyl phenidete hydrochloride in tablet formulation. The result

for Methyl phenidete hydrochloride was comparable with a corresponding labelled amount (Table 6). The absence of additional peaks indicates no interference of the excipients used in the tablets.

Formulation

Formulation	Tablet dosage	Sample concentration	Amount of drug estimated
RITALIN CAOSULE	20mg	40ppm	99.55

Table 8: Tablet estimation of Methyl phenidete hydrochloride

Conclusion

A validated RP-HPLC method has been developed for the determination of Methyl phenidete hydrochloride in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic run time of min allows the analysis of a large number 10 of samples in short period of time. Therefore, it is suitable for the routine analysis of Methyl phenidete hydrochloride in pharmaceutical dosage form.

References:

1 "Ritalin & Cocaine: The Connection and the Controversy". Learn.genetics.utah.edu. Retrieved on 2011-10-16.

2 Mary Ann Boyd (2005). *Psychiatric nursing: contemporary practice.* Lippincott Williams & Wilkins.. Retrieved 30 April 2011.

3 Peter Doskoch (2002). "Why isn't methylphenidate more addictive?". *NeuroPsychiatry Rev.* **3** (1): 19. Archived from the original on 2009-03-30.

4 Markowitz JS, Logan BK, Diamond F, Patrick KS (1999). "Detection of the novel metabolite ethylphenidate after methylphenidate overdose with alcohol coingestion".*Journal of Clinical Psychopharmacology* **19** (4): 362–6..

5 Steele M, Weiss M, Swanson J, Wang J, Prinzo RS, Binder CE (2006). "A randomized, controlled effectiveness trial of OROS-methylphenidate compared to usual care with immediate-release methylphenidate in attention deficit-hyperactivity disorder" (PDF). *Can J Clin Pharmacol* **13** (1): e50–62. .

6 Gilmore A, Milne R (2001). "Methylphenidate in children with hyperactivity: review and cost-utility analysis".*Pharmacoepidemiol Drug Saf* **10** (2): 85–94.

7 Mott TF, Leach L, Johnson L (2004). "Clinical inquiries. Is methylphenidate useful for treating adolescents with ADHD?". *The Journal of Family Practice* **53** (8): 659–61. .

8 Vitiello B (2001). "Psychopharmacology for young children: clinical needs and research

opportunities".*Pediatrics* **108** (4): 983–9. .

9 Hermens DF, Rowe DL, Gordon E, Williams LM (2006). "Integrative neuroscience approach to predict ADHD stimulant response". *Expert Review of Neurotherapeutics***6** (5): 753–63.

10 Fone KC, Nutt DJ (2005). "Stimulants: use and abuse in the treatment of ADD". *Current Opinion in Pharmacology* **5** (1): 87–93.

11 Capp PK, Pearl PL, Conlon C (2005). "Methylphenidate HCl: therapy for attention deficit hyperactivity disorder".*Expert Rev Neurother* **5** (3): 325–31

12 Greenfield B, Hechman L (2005). "Treatment of attention deficit hyperactivity disorder in adults". *Expert Rev Neurother* **5** (1): 107–21.

13 Swanson JM, Cantwell D, Lerner M, McBurnett K, Hanna G (1991). "Effects of stimulant medication on learning in children with ADHD". *J Learn Disabil* **24** (4): 219–30, 255.

14 Joseph Biederman, MD, Timothy Wilens, MD, Eric Mick, ScDv, Thomas Spencer, MD, Stephen V. Faraone, PhD (1999). "Pharmacotherapy of Attention-deficit/Hyperactivity Disorder Reduces Risk for Substance Use Disorder".*Pediatrics* **104** (2): 20ff.

15 Schachter HM, Pham B, King J, Langford S, Moher D (2001). "How efficacious and safe is short-acting methylphenidate for the treatment of attention-deficit disorder in children and adolescents? A meta-analysis". *CMAJ* **165** (11): 1475–88.

16 Morgan AM (1988). "Use of stimulant medications in children". *Am Fam Physician* **38** (4): 197–202.

17 Stevenson RD, Wolraich ML (1989). "Stimulant medication therapy in the treatment of children with attention deficit hyperactivity disorder". *Pediatr. Clin. North Am.* **36** (5): 1183–97.

18 Kidd PM (2000). "Attention deficit/hyperactivity disorder (ADHD) in children: rationale for its integrative management" (PDF). *Altern Med Rev* **5** (5): 402–28.

19 Fry JM (1998). "Treatment modalities for narcolepsy".*Neurology* **50** (2 Suppl 1): S43–8. .

20 Mitler MM (1994). "Evaluation of treatment with stimulants in narcolepsy". *Sleep* **17** (8 Suppl): S103–6.

