Academic Sciences

International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 7, Issue 3, 2015

Original Article

SPECTROPHOTOMETRIC DETERMINATION OF MEPHEDRONE IN A BULK FORM

VAISHALI PARASTEKAR, VASANT BARHATE*

V. E. S College of Arts, Science and Commerce, Sindhi Society, Chembur, Mumbai 400071
Email: vasantbarhate@yahoo.co.in

Received: 22 May 2015, Revised and Accepted: 31 May 2015

ABSTRACT

Objective: Two simple and sensitive Spectrophotometric methods are developed for estimation of Mephedrone.

Methods: The first method is based on oxidation of the drug with alkaline Potassium Permanganate at room temperature (25± °c). The increase in absorbance of colored Manganate ions was measured at 610 nm. The second method is based on the oxidation of the drug with 1, 10–phenanthroline producing red colored chromogen which is measured at 510 nm.

Results: All parameters affecting the developments of the color were investigated and the conditions were optimized. Under the optimum condition used, Beer's law was obeyed in the concentration range 40-160 µg/ml and 80-220 µg/ml for method A and B respectively. Molar absorptivity was found to be 4.60×10^2 L. mole^{-1} cm^{-1}and 3.89×10^2 L. mole^{-1} cm^{-1} respectively.

Conclusion: The proposed methods are well suited for determination of Mephedrone in bulk samples.

Keywords: Mephedrone, Spectophotomerty, Oxidation, Potassium permanganate, 1, 10-phenanthroline.

INTRODUCTION

Mephedrone, also known as 4-methylmethcathinone (4-MMC) or 4-methylephedrone is a synthetic stimulant drug of amphetamine and cathinone class. Slang names include drone, M-CAT and Meow Meow. It is chemically similar to the cathione compounds found in khat plants of eastern Africa. It comes in the form of tablets or a powder, which user can swallow, snort or inject, producing similar effects to MDMA, Amphetamines and Cocaine. Mephedrone is one of the designer drug. This is primarily developed to avoid being controlled by laws against illegal drugs, thus giving them the label of designer drugs.

Mephedrone causes euphoria, stimulation, an enhanced appreciation for music, an elevated mood, decreased hostility, improved mental function and mild sexual stimulation. These effects are similar to the effects of cocaine, amphetamines and MDMA. Like other drugs even Mephedrone has side effects like loss of appetite, muscle clenching and tremors, headache, anxiety, elevated blood pressure, chest pain, irregular heartbeat, difficulty in urinating, changes in body temperature and blue/cold fingers [1]. Various methods of analysis and detection such as LC [2, 3].

LC-MS [4, 5], MS [6], HPLC after liquid extraction [7] are available in literature.

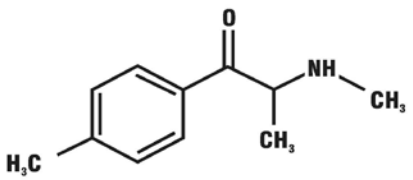

Fig. 1: Structure of Mephedrone

The present paper describes two visible spectrophotometric methods using $KMnO_4$ as an oxidizing agents, for method A & Fe (III) 1,10-phenanthroline for method B. Simplicity, Sensitivity, wide linear range, mild experimental conditions and above all the cost effectiveness characterize the proposed methods. The methods were found to possess adequate accuracy and precision. The aim of the present work is to develop simple method for the determination of Mephedrone.

MATERIALS AND METHODS

A) Apparatus

A model Specord 600 spectrophotometer with 1 cm matched quartz cell was used for all absorbance measurements.

B) Reagents and materials

All chemicals used were of analytical grade reagent and double ionized distilled water was used to prepare all solutions. Potassium permanganate (5×10^{-3}) mol/lit was prepared by dissolving 0.079 gm of chemical, Merck, Mumbai) in water and diluting to 100 ml and standardized using H. G. Brights procedure (A. I Vogel, 3rd edition, 1961, Pg. No-280) Sodium hydroxide solution (0.5 mol/lit) was prepared by dissolving the chemical (Merck, Mumbai, India) in water.

Fe (III) solution is prepared by dissolving 54 mg of anhydrous Ferric chloride in 100 ml of double deionised distilled water. 0.2 % (w/v) O-Phenanthroline is prepared by dissolving 200 mg of reagent in 100 ml of double ionized distilled water. 1.25 % (v/v) O-Phosphoric acid solution is prepared by diluting 1.25 ml of A. R grade O-Phosporic acid to 100 ml with D/W.

Mephedrone certified to be 99.5% pure was kindly provided by F. D. A, Mumbai for research work, and a solution of 2000 µg/ml and 1000 µg/ml was prepared from it. 100 mg and 200 mg of Mephedrone are accurately weighed and transferred into 100 ml standard flask respectively and dissolved with double ionized distilled water and made up to mark with constant shaking to obtain 1000 µg/ml and 2000 µg/ml working standard solutions.

Method-A:-Different aliquots of std solution (1.0 to 4.0 ml,1000 µg/ml) of the pure Mephedrone were transferred into a series of 25 ml calibrated flask by means of micro burette. A volume of 2 ml of (0.5 mol/lit NaOH was added to each flask accurately. To each flask was added 2 ml of (5×10^{-3}) mol/lit $KMnO_4$. The Final volume made to 25 ml with D/W. The flask was kept aside for 10 minutes with occasional shaking. The absorbance was recorded at 610 nm against the reagent blank.

Method-B:-Into a series of 25 ml calibrated flask (1.0 to 2.75 ml, 2000 µg/ml of pure mephedrone were buretted. A volume 3.0 ml of 0.54% w/v of Fe (III) solution was added to each flask and 2 ml of 0.2 % w/v of O-phenanthroline was added successfully. The flask

was kept on a boiling water bath for 30 minutes. The flask were removed and cooled to room temperature. 2 ml of O-Phosphoric acid is added and volume is made to 25 ml. The absorbance of the colored product is measured against a reagent blank prepared similarly. Maximum absorbance is found to be at 510 nm.

Assay procedure for bulk drug

100 mg and 200 mg of a bulk drug was accurately weighed and transferred into a 100 ml calibrated flask respectively. 40 ml of distilled water was added and shaken for 20 minutes. Then the volume was made to 100 ml with D/W in both flasks. Mixed well and filtered using whatman filter paper no-42. Convient aliquot, (1.0 to 3.5 ml) and (1 to 2.5 ml) was subjected to analysis by the procedure described under Method A and Method B respectively.

RESULTS AND DISCUSSION

The reaction between Mephedrone and $KMnO_4$ in alkaline solution yields a green color as a result of Manganate species [8, 9] which absorbs at 610 nm. The intensity of the color product increases gradually reaching its maximum after 10 minutes, (fig. 2) when it remains stable for at least ½ an hour (fig. 3). As the intensity of color increases with time, it was deemed useful to elaborate a method for determination of Mephedrone in bulk. The reaction was investigated under various conditions of reagent concentration and alkalinity. Water was used to dissolve the drug since $KMnO_4$ oxidizes with the production of green Manganate ions. At room temperature the reaction increased substantially with time, as revealed by the intensification of the developed color and subsequent increase in the slope of the calibration graph indicating high analytical sensitivity. The second method is based on the oxidation of drug with Fe^{+3}, 1-10 Phenanthroline producing red chromogen which absorbs at 510 nm. The intensity of color increases gradually reaching maximum after heating and remains stable for 24 hr (fig. 5).

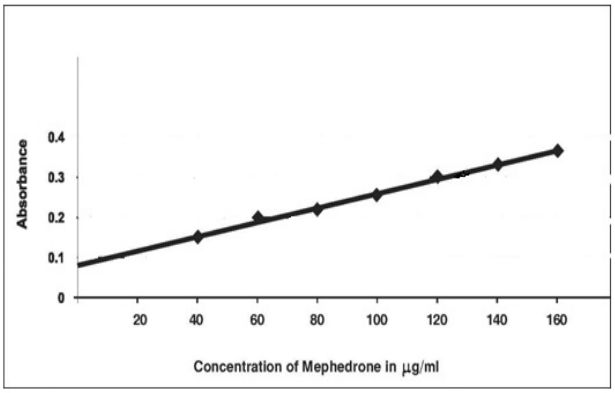

Fig. 2: Calibration curve for method-a

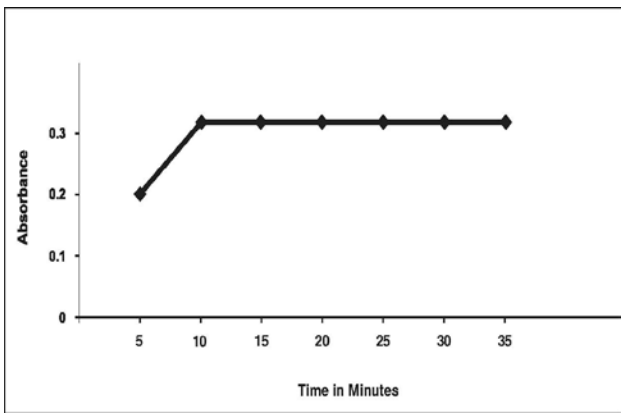

Fig. 3: Stability of complex in method-a

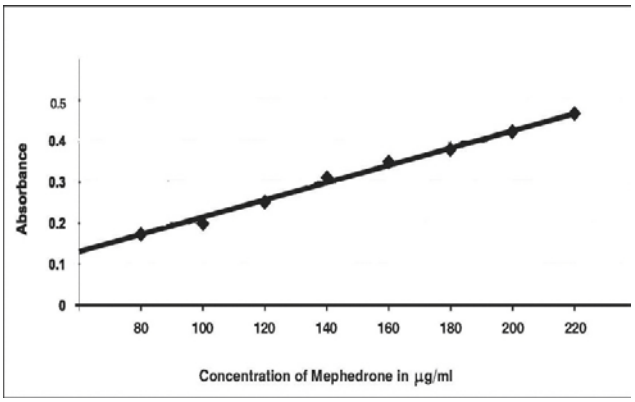

Fig. 4: Calibration curve for method-b

Optimization of parameters

Method-A

All the optimization parameters are estimated at room temperature for method A. The investigations were carried out to establish the most favorable conditions for the formation of colored product. The influence of concentration as well as the volume of the reagent on the reaction has been studied. Different concentration and different volume were tried for all the reagents by varying one parameter at a time.

1) The influence of $KMnO_4$

The reaction rate and absorbance increases with increasing $KMnO_4$ concentration. The absorbance was studied in the range $1\times10^{-4}$to 1×10^{-3} mol/l keeping all other parameters constant. It was found that 7.5×10^{-4} mol/l $KMnO_4$ is the optimum concentration for the absorbance of Mephedrone as shown in (fig. 6). The effect of the color development was investigated by adding different volume (0.1-2.0 ml) of 7.5×10^{-4} mol/l, potassium permanganate to a drug. The maximum absorbance of the green color was attained with 1.5 ml of the coloring reagent, and remained constant even when higher volumes were added (fig. 7) Therefore; 2 ml of the reagent was used for the experimental investigations.

Two ml of $KMnO_4$ must be accurately added in all the reaction flask since $KMnO_4$ absorbs maximally at the analytical wavelength, and small changes in the volume of $KMnO_4$ have a critical effect on the absorbance reading.

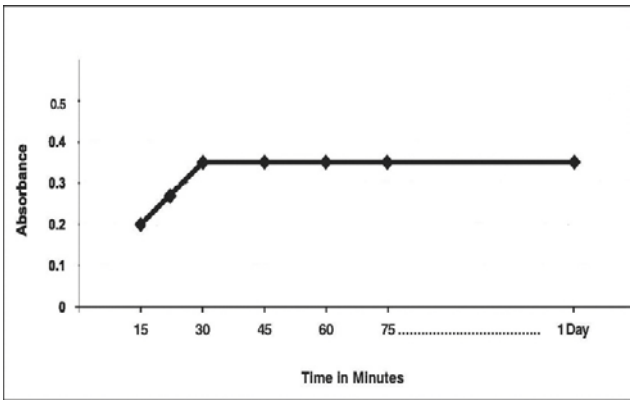

Fig. 5: Stability of complex in method-b

2) Influence of the NaoH

The reaction rate and absorbance increases with increasing $KMnO_4$ concentration on the formation of MnO_4^{-2} was also examined at constant concentration of drug, Permanganate ion and varying volume (0.2-2.0 ml) of O.5 Mol/l NaOH at 25 °C. The optimum

absorbance was obtain with 1.5 ml of 0.5 mol/l NaOH after which increase in volume of NaOH caused no changed in absorbance. Hence 2 ml of 0.5 Mol/l NaOH was used throughout the experimental investigation (fig. 8).

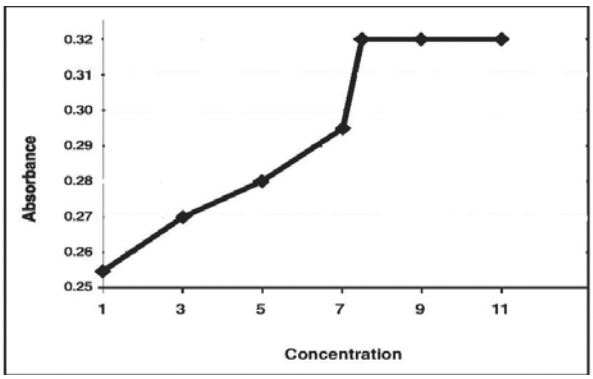

Fig. 6: Effect of the concentration ranges 1×10^{-4} to 1×10^{-3} of $KMnO_4$ on the intensity of the color produced during the reaction (Mephedrone 120 µg/ml: 1 ml of 0.5 mol/l NaOH)

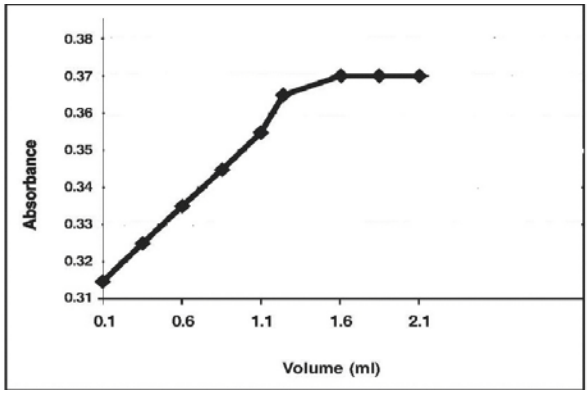

Fig. 7: Effect of the volume of 2 mol/l $KMnO_4$ ON the intensity of the color produce during the reaction (Mephedrone 120 µg/ml: 2 ml of 0.5 mol/l NaOH)

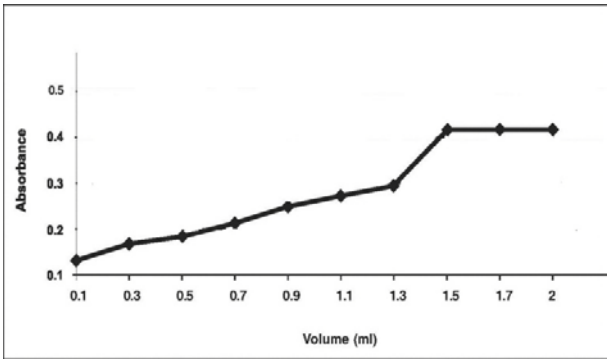

Fig. 8: Effect of the volume of 0.5 mol/l NaOH on the intensity of the color produced during the reaction (Mephedrone 120 µg/ml: 2 ml of 7.5 mol/l $KMnO_4$

Method-B

Ferric salts play a prominent role in spectrophotometric determination of many pharmaceutical drug substances acting as an oxidant [10, 11]. The $Fe^{+3/[1,10]}$ O-phenanthroline (Fe^{+3}/Phen) system is valuable reagent for any analytes with reducing properties, because the final product is intensely colored and extractable chelate $[Fe(phen)^3]^{2+}$. Mephedrone undergo oxidation by FPL reagent in a weakly acidic medium, forming an orange red colored complex with absorption maximum at 510 nm. The optimum reaction parameters were established via a number of preliminary experiments.

Ferric salt converts into a ferrous salt upon oxidation and can be easily detected by the usual reagent O-Phenanthroline. The reaction product is tris complex of Fe (III), well known as ferroin. The colored product of the reaction is given below in fig.-9.

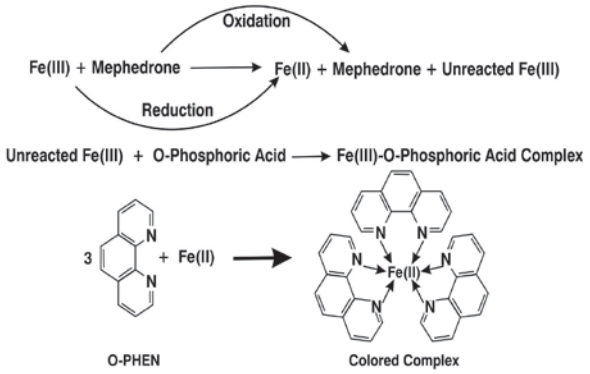

Fig. 9: Reaction of Mephedrone with $Fe^{+3/[1,10]}$O-phenanthroline

The color intensity of the formed complex $[Fe(phen)^3]^{2+}$was measured against different concentration of reagent ranged from 0.25 to 3.0 ml. As shown in fig. 10. Reagent ranging from 2.0 to 3.0 ml gave the maximum absorbance values hence 3.0 ml of the reagent was used throughout the study.

The formation of colored complex was slow at room temperature and required longer time for maximization because of kinetic hindrance. The reaction was accelerated by heating for 25 minutes, at higher temperature 60 ° to 100 ° C using thermostatically controlled water bath. It was observed that the maximum absorbance was obtained upon boiling. Different boiling times were then further investigated over time intervals ranged from 5 to 30 minutes. The maximum color intensity was obtained after boiling the reaction mixture for 25 minutes. Thus, boiling for 30 minutes chosen as optimum boiling time to assure complete reaction. The absorbance of the resulting colored product was stable at room temperature for more than 24 hrs.

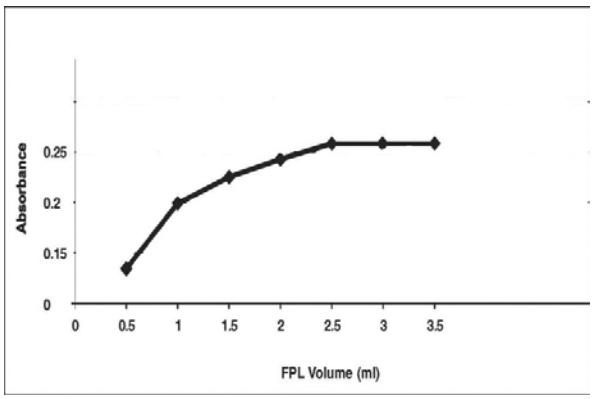

Fig. 10: Effect of different volume of 0.01 M FPL on the development reaction product with Mephedrone

1) **Influence of the O-Phosphoric acid**

Effect of varying concentration of O-Phosphoric acid on the absorbance of colored product was studied. The optimum absorbance was obtained with 1.0 ml of 1.25% of O-Phosporic acid hence 2.0 ml of 1.25 % O-Phosporic acid was used throughout experimental investigation. (fig. 11)

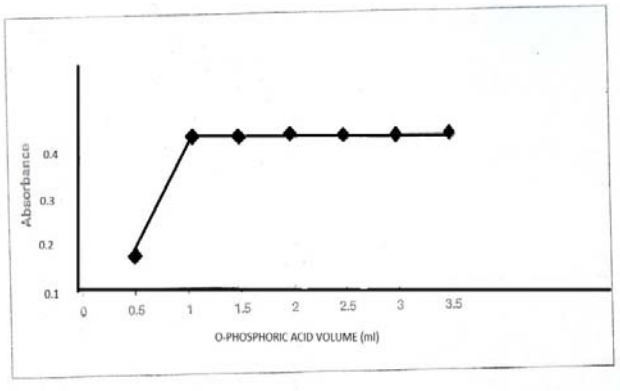

Fig. 11: Effect of different volume of 1.25 (v/v) O-phosphoric acid on development of reaction product with Mephedrone

For method a

The optimum concentration of $KMnO_4$ was 5×10^{-3} M.

And optimum volume is 2 ml. The absorbance was measured 10 minutes after the final dilution resulted product is stable for 30 minutes thereafter.

For method b

The optimum concentration of Fe (III) solution is 0.054 %,that O-phenanthroline is 0.2 % (w/v) and of O-phosporic acid is 1.25% (v/v).

Validation of methods

The two proposed methods were validated based on linearity and inter-day and intraday precision, accuracy, specificity and robustness.

Linearity was evaluated by calculation of the regression equation over the ranges given in Table-1. The table also shows the detection limits, slopes, intercepts and correlation coefficient obtained by linear least squares treatment of the results, standard deviation of slopes (Sb) and intercepts (Sa) on the ordinates, and standard deviation of the residuals (Sy/x)

The intraday and inter-day accuracy and precision for the proposed methods were examined by analysis of samples of Mephedrone on two different concentrations. Intraday precision was assessed by five concentration determination in one day. Inter-day precision was assessed by determination of each concentration on three separate days. Repeatability and reproducibility in two proposed methods were fairly good, as indicated by small values of standard deviation (SD), relative standard deviation (RSD)

Robustness of the two methods is demonstrated by the consistency of the absorbance intensity with minor changes win experimental variables, such as changing the reaction times, changing heating time, and the reagent volume. The minor changes expected to take place during the course of the operation of the method did not adversely affect the absorbance intensity.

Table 1: Analytical parameters for spectrophotometric determination of Mephedrone in bulk form by applying Method A and B

Parameters	Method A	Method B
Maximum wavelength (nm)	610 nm	510 nm
Linearity Range (µg/ml)	40-160	80-220
Intercept (a)	0.09	.12
Std deviation of intercept (Sa)	.011	.02
Slope (b)	1.73×10^{-4}	2.13×10^{-3}
Std deviation of slope (Sb)	5.76×10^{-4}	9.04×10^{-4}
Correlation Coefficient (r)	.9961	.9974
Molar absorptivity L. $mole^{-1}$. cm^{-1}	4.60×10^{2}	3.89×10^{2}

Table 2: Sample analysis of bulk drug of Mephedrone analyzed by the proposed method as per the procedure described earlier

Bulk drug of Mephedrone	Mephedrone found	
	Method-A	Method-B
	98.9 %	99.03 %

CONCLUSION

Two simple and sensitive spectrophotometric methods were developed for the determination of mephedrone in bulk form. The methods are free, rigid over experimental conditions and are characterized by wide linear dynamic ranges and has high sensitivity and employ inexpensive and easily available chemicals. The low detection and quantitation limits, simplicity and selectivity make the method suitable for quqlity control in pharmaceutical industry for routine analysis.

ACKNOWLEDGEMENT

The authors are greatly thankful to Dr. Sandeep Chetti (DFSL, Mumbai) and FDA laboratories, Mumbai for providing the drug sample for research work and guidance.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Adam Winstock, Luke Mitcheson, John Ramsey Susannah Davies, Malgorzata Puchnavewicz, John Marsden. Mephedrone use, subjective effects and health risks; Society for the study. Addiction 2011;106(11):1991-6.
2. C Rambabu, S Venkata Rao, G Ramu, A Biksham Babu. Quantitative analysis of Mephedrone in bulk and pharmaceutical formulations by reverse phase high performance liquid chromatography. Rasayan J Chem 2010;3(4):796-9.
3. Eman Y Santali, Anna-Karnia Cadogan, Niamh Nic Daeid, Kathleen A. Sarage, Oliver B. Sulclifee. Synthesis, full chemical characterization and development of validate d methods for the quantitation of (±)-4 methylmethacathinone (Mephedrone). J Pharm Biomed Anal 2011;56(2):246-55.
4. Peter Jankovics, Andras Varadi, Laszio Tolayesi, Szilvia Lohner Julia Nemeth Palotas, Hilda Koszegi szalai. Identification & characterization of the new designer drug 4'-methylmethacathinone (4-MEC) and elaboration of a novel liq chromatography-tandem mass spectroscopy [LC-MS]. Forensic Sci Int 210;(1-3):213-20.
5. Emmislivka, Characterization of (MDPV) & Mephedrone in "Legal High" products by chemical colour test, microcrystalline tests with confirmation by LC-MS, The Center For Forensic Science Research & Education, Willow curve, PA. ARCADIA university; 2011.
6. Syeda A Shah, James Barker, Andrea Petroczi, Paul Cross, Roland Archer. Quantitative analysis of Mephedrone using Liquid chromatography tandem mass spectroscopy; Application to human hair. J Pharm Biomed Anal 2012;6:64-9.
7. El Shamaileh Majed, Gonzalez-Rodriquez, Jose, Baron, Mark, Croxton, *et al*. A rapid HPLC-DAD method for the simultaneous analysis of mephedrone & methcathinone after liquid-liquid extraction from serum & whole blood matrices; IALM 2012, 22nd congress of the international Academy of legal Medicine, Turkey; 2012.
8. Aftab Aslam Parwaz Khan, Ayaz Mohd, Shaista Bano, KS Siddiqi, Abdullah Mohammed Asiri. Spectrophotometric methods for the determination of Ampicillin by $KMnO_4$ and 1-chloro-2, 4-dinitrobenzene in pharmaceuticals. Arabian J Chem 2015;8(2):255-63.
9. Kalsang Tharpa, Kanakapura Basavaiah, Nagaraju Rajendraprasad, Kanakpura Basavaiah Vinay, Salmara Ganeshbhat Hiriyanna. Sensitive spectrophotometric assay of simvastin in pharmaceuticals using permanganate. Braz J Pharm Sci 2010;46:1.

10. Mohamaed, El-Ghaffar, Dina El Sherbiny, Dalia El-wasseef, Saadia El-Ashry. Spectrophotometric methods for determination of Ritodrine in bulk and in pharmaceutical dosage form. J Food Anal 2008;16(2):26-35.

11. Biksham Babu, G Ramu, S Venkata Rao, T Neeharika, C Rambabu. Spectrophotometric determination of an antiretroviral drug stavudine in bulk and pharmaceutical formulations: A. Rasayan J Chem 2011;4(2):33.