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QUANTITATIVE ANALYSIS OF MEPHEDRONE IN BULK AND PHARMACEUTICAL FORMULATIONS BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRCT

A rapid, precise, economical and accurate reverse phase liquid chromatographic method for estimation of Mephedrone in bulk and formulations was developed and validated. The Chromatographic resolution of Mephedrone was achieved using Acetonitrile: 0.1M Ammonium Acetate Buffer PH~ 4, (40: 60 V/V) as a mobile phase UV detection at 232 nm and C18 column .flow rate 1ml/min. Recovery of Mephedrone from its formulation dosage form was greater than 99.8% . Calibration curve was linear (r2 = 0.9992) over Mephedrone concentration ranging From 20 to 120 µ g /ml. the method has an accuracy of greater than 99% . The LOD and LOQ of the drug were 0.80339 µ g /ml and 2.43450 µ g /ml respectively. Results were validated statistically and recovery studies were found to be satisfactory.

Keywords: Mephedrone, Entactogen drug , RP-HPLC, Assay, LOD, LOQ

INTRODUCTION

Mephedrone is also known as Methyl Methcathinone, Is a synthetic stimulant And entactogen drug and it is a synthetic drug which is manufactured in china Based on the cathinone compounds found in the KHAT plant of eastern Africa. We brought the both samples standard and formulation from china internet Source, It is available in both capsule and powder form as in the name of Plant Food, salt bath. There are few papers published reporting analytical methods for mephedrone Two of them are employed GC-MS with split-split less injection (2µl injected)[1] and MSD (EI, 70ev, TIC mode scanning M/z 40-800, temperature program 90ºC, 15ºCAnd 280ºC. Two of them are available to describe the pattern and clinical features Of toxicity, employing a basic liquid-liquid extraction followed by Penta Fluropropionic Anhydride derivatization, was used to isolate mephedrone from Both blood and urine Specimens. The derivatized extracts were analyzed by gas Chromatography mass spectrometry (GC-MS) operating in full-scan mode[2]. Quantitative analysis of Mephedrone was performed by GC-MS operating in Selective ion monitoring mode Using methamphetamine-d_{14} as an internal Standard. Mephedrone was confirmed in the decedent's blood and urine at 0.50 and 198 mg/L respectively[3]. But none of them are employed an economical, precise and accurate RPHPLC method, so we here present a new method for determination of mephedrone in bulk and Pharmaceutical dosage forms which utilizes a very cheap solvent system On Hypersil ODS C 18 analytical columns. This kind of method effective to produce Better retentions, very sharp and symmetrical peak shapes and exhibit very good sensitivity for Mephedrone. The structure of Mephedrone is given below.

Instrumentation

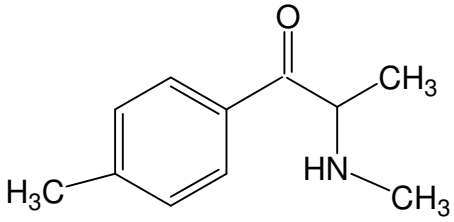
Quantitative HPLC was performed on a binary gradient HPLC with Shimadzu LC20AT and LC20AT VP series HPLC pumps, with a 20µl injection of sample loop (manual), and SPD20 A VP UV –Visible Detector. The out put signal was monitored and integrated using Shimadzu Class VP version 6.12 SP1 software. Hypersil, thermo, C18 (250 x 4.6µ, 5µ) column was used for Separation**.**

Standards and Reagents

Mephedrone and its formulation capsules were purchased from internet sourcing from china and drotovirine is gifted by Chandra labs .Acetonitrile HPLC grade, Ammonium Acetate were purchased from Merck

Preparation of Standard Drug Solution

50mg each of Mephedrone and Drotavirine (IS) were accurately weighed and transferred in two separate 50 ml of volumetric flask containing 25ml of mobile phase and sonicated for 10 min, diluted with mobile phase up to the lower meniscus mark and filtered it through 0.45µmemberane filter to get the stock solution of 1mg /ml



EXPERIMENTAL

Chromatographic Conditions

The mobile phase used in this study was a mixture of Acetonitrile and Phospharicacid (buffer PH~4) 40:60 V/V, then the content was solicited for 30 min for degassing purpose and then filtered through 0.45 µ (pore diameter) filter . The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 1.0ml/min. the eluents were monitored at 230 nm. The column temperature was maintained ambient through out the experiment.

Calibration of Standards

Calibration standards were prepared by spiking working standard into mobile phase containing 25ml volumetric flask to yield concentrations of 20. 40. 60, 80, 100, 120µg/ml and the final volume was made up to the mark. The represented data was shown in Table- 1. Chromatograms are obtained 20 µl aliquot was injected into the analytical column. The resultant peak areas of the drug and internal standard were measured. Calibration curve was plotted between peak area ratios of drug and internal standard against concentration of the drug.

RESULTS AND DISCUSSION

The standard solution of Mephedrone was injected into the HPLC system. The system suitability parameters were evaluated and found to be within the limits. The USP tailing factor, Number of Theorical Plates, and %RSD were found to be 0.941, 2887, and 0.299 respectively. Linearity of the detector response to the concentration of the standard drug was established by plotting a graph to concentration of Mephedrone versus average area of two peak areas and determining regression parameters. A series of six solutions of were prepared in the concentration range of about 20.0µg/ml to 120µg/ml corresponding to 20% to 120% of target concentration. Each solution was injected into the system and recorded the chromatogram under the standard chromatographic conditions. A graph was plotted to concentration in µg/ml on X-axis versus response on Y-axis (Fig.-2). The detector response was found to be linear with a correlation coefficient of 0.9996. The results were summarized in Table-1.

Precision and accuracy of the developed method was studied by preparing three different concentration solutions of Mephedrone with in the linearity limits equivalent to about 80%, 100%, and 120% of the target concentration the drug. These solutions were prepared in triplicate for each spike level and assayed as per standard method. The % recovery was calculated and found to be within limits. The results were summarized in Table-2and Table-3. Specificity and selectivity of the method was assessed by preparing a drug concentration 100 µ g/ml from pure drug stock and commercial sample stock in selected mobile phase and analyzed. The HPLC chromatograms were recorded for the drug matrix showed almost no other peaks within a retention time range of 7 min (Figure 3). Thus the HPLC method developed in this study is selective for Mephedrone.

Recovery of mephedrone from its formulations was studied by preparing finely powdered formulation dosage and accurately weighed sample of formulation equivalent to 50 mg Mephedrone was extracted with Acetonitrile in a 50ml volumetric flask using ultra sonicator. This solution was diluted with mobile phase, so as to obtain a concentration in the range of linearity previously determined. An aliquot (100 µg /ml) of the internal standard was added to the sample solution prior to the dilution. All determinations were carried out in five replicates. The represented data was shown in Table-5.

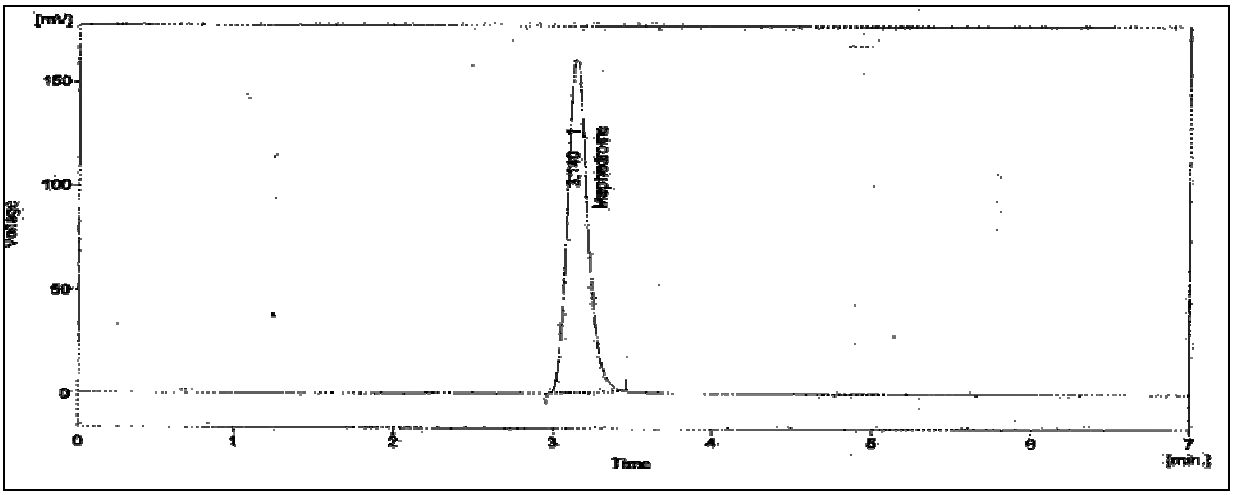


Fig-1: HPLC chromatogram of Mephedrone (standard)

Table-1: System Suitability

S.No.	Suitability Parameter	Value of the Parameter
1	Number of Theoretical Plates	2887
2	Tailing Factor	
3	%RSD	0.299

Table-2: Linearity of the Detector Response

Concentration µg/ml	Peak Area Ratio	Statistical Parameters	
20	374.766	Slope	17.639
40	696.456	Intercept	22.33
60	1087.744	Correlation Coefficient	0.9994
80	1416.262	LOD	0.80339
100	1746.432	LOQ	2.4345
120	2112.450		

Table-3: Precision

Concentration µg/ml	Peak Area	%RSD[a]
80	1413.821	0.299

[a]Each value is average of five determinations

Table-4: Accuracy Studies

Mixture of pure and Formulation	Concentration of Formulation	%Recovery of the Pure Drug	%RSD[b]
80%	80 µg/ml	100.0232	0.025234
100%	100 µg/ml	99.9919	0.017496
120%	120 µg/ml	100.0046	0.019342

[b]Each is average of five determinations

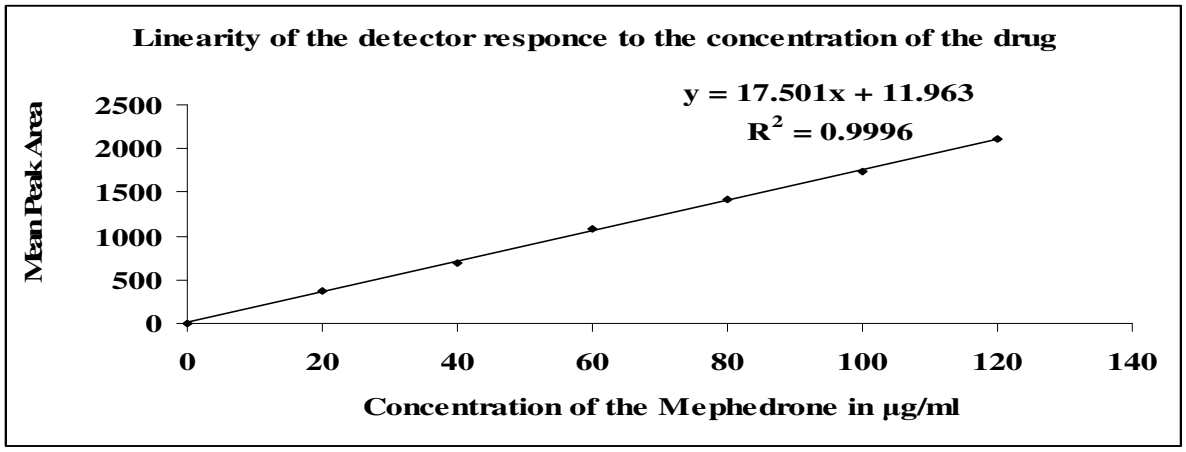


Fig.-2: Linearity plot of Mephedrone Drug

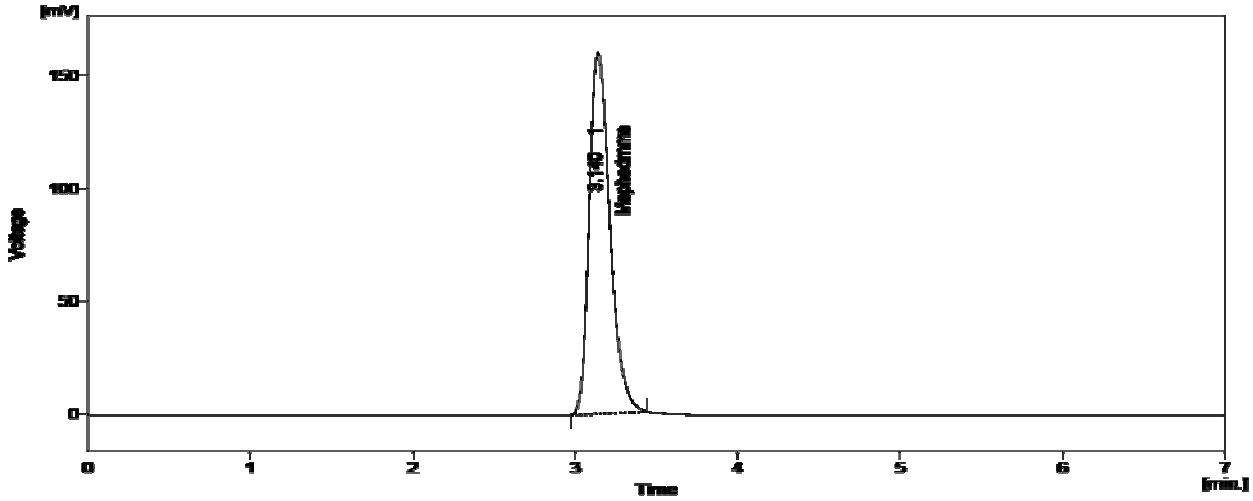


Fig.-3: Chromatogram of Mephedrone - Formulation

Table-5: Amount of Mephedrone in formulation dosage By HPLC Method

[c]Each value is the average of five determinations

Type of Formulation	Labeled Amount (mg /tablet)	Weight Found (Mean ± SD)	% Recovery ± %RSD[c]
Tablet	100	99.48±0.371	99.48±0.373

CONCLUSIONS

The results obtained from these studies are well fit into the standard specifications stipulated by the regulatory agencies. The method is able to reproduce the results consistentantly and satisfied, hence this can be conveniently used in the pharmaceutical manufacturing and formulation environment for equipment cleaning certification.

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