HPTLC-Densitometric Method for Estimation of in Bulk, Pharmaceutical Formulations

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Abstract: A simple, sensitive and precise HPTLC (high performance thin layer chromatography) method has been developed for quantitative estimation of ketamine in bulk and pharmaceutical formulations. The separation of ketamine was achieved over HPTLC glass plate of silica GF-254 by using cyclohexane:toluene:diethylamine (5:3:2 v/v) solvent system. Densitometric evaluation was carried out in the UV region at wavelength of 254. The method was found to be accurate (98.92–100.26%) and precise (CV = 0.43–0.67 for intra-day and CV = 0.58–1.45 for inter-day) within the corresponding linear range. The limit of detection (LOD) and limit of quantification (LOQ) of sample have been found to be 100 ng/spot and 303 ng/spot, respectively.

Keywords: Date rape drug, Densitometric method, Drug of abuse, General anesthetic, Hallucinogen, HPTLC, Ketamine.

I. Introduction

Ketamine [2-(2-chlorophenyl)-2-(methylamino)cyclohexanone hydrochloride, 1] is a rapid acting anesthetic used during surgical procedures in both animals and humans [1]. The drug is sometimes misused in a sexual assault and hence referred as date-rape drug. The compound is odorless and tasteless and can be added to beverages, without being detected, to induce amnesia [1]. It is available in powder, liquid and solid dosage forms, and for abuse purposes it is smoked, snorted, injected, or taken orally [2]. Ketamine induces amnesia very rapidly and causes minimal adverse effect on respiratory or central nervous system. So it is often considered as safe and clean drug by first time users. This is the reason why it has emerged as the fastest growing drug of abuse in recent years [3-6]. More recently, it is being increasingly encountered as an added component in ecstasy-type tablets which include amphetamine type stimulants and phencyclidine (PCP) [7]. The compound has been analyzed and quantified using titrimetric [8] infrared spectroscopy [9], gas chromatography/mass spectrometry (GC/MS) [10] and ultraviolet spectroscopy [11, 12]. But, due to its growing misuse, it is required to develop a rapid and efficient method for quantification. The present work is a step towards development of a simple, sensitive and precise quantification method in bulk and pharmaceutical formulations.

II. Experimental

A. Materials

Ketamine was obtained from Indian Pharmacopoeia Commission, Ghaziabad, and some injectible formulations of it were procured from the local market. All chemical and reagents used were of analytical grade and were purchased from reputed companies.

B. Preparation of Standard Stock Solution

Ketamine (5 mg) was weighed and transferred to a volumetric flask (10 mL) and subsequently methanol (5 mL) was added. The flask was shaken, and volume was made up to the mark with methanol to give a solution containing 500 ng/µL of Ketamine.

C. Sample Preparation

The solution of Ketamine (1 mL) purchased from open market was transferred to a volumetric flask (10 mL) followed by the addition of methanol (5 mL), flask was shaken and volume was made up to the mark with methanol. The above solution was filtered through Whatman filter paper (0.45 µm), this would give a solution containing 500 ng/µL of Ketamine (solution A). This solution was used for the estimation of Ketamine.

D. HPTLC Preparation

The samples were spotted in the form of bands of width 6 mm with a Camag Linomat V, Automatic Spotter equipped with a 100 µl syringe on precoated silica gel aluminum plate 60 F 254 (20×10 cm with 250 µm thickness; E. Merck Germany). A constant application rate of 150 µl sec^{-1} was employed and space between two bands was 5 mm. The slit dimension was kept at 5.00 mm × 0.45 mm with 20 mm sec^{-1} scanning. The mobile phase consisted of cyclohexane:toluene:diethylamine

(5:3:2 v/v). Methanol was used as diluents for standard and sample preparation. Linear ascending development was carried out in twin trough glass chambers saturated with the mobile phase. The optimized chamber saturation time phase was 20 min at room temperature. The length of the chromatogram run was 7 cm (Fig. 1). Subsequent to the development, HPTLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed on CAMAG HPTLC Scanner –III (Camag Muttenz, Switzerland) in the absorbance mode at 254 nm (Fig. 1). The source of radiation utilized was deuterium lamp.

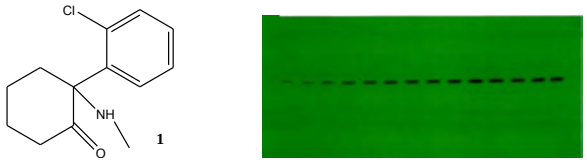

Fig. 1. Developed HPTLC plate of ketamine

E. Estimation of Ketamine in Pharmaceutical Preparation

By using prepared solution A, a quantity equivalent to 6 µL was applied on pre-washed TLC plate, developed in the above mobile phase, dried in air, and analyzed densitometry. From the peak area obtained in the chromatogram (Fig. 2), the amount of the drug was calculated.

F. Calibration Curve for the Ketamine (400 to 1200 ng/spot)

Stock solution was filled in the syringe and under nitrogen stream by a semiautomatic sample applicator; it was applied in the form of a band of drug on a plate having concentrations of 400 to 1200 ng/spot of Ketamine. The plate was developed using a mixture of cyclohexane: toluene: diethylamine (50:30:20 v/v) at 27 ± 1°C and dried in air. The developed plate was subjected to densitometric measurements in absorbance mode at wavelength 254 nm using TLC Scanner III (Fig. 1). Plots of peak area *vs* concentration for Ketamine were obtained (Fig. 3). Spectra of Ketamine bands were recorded in the range of 200–400 nm, and purity of chromatographic peak was checked by scanning individual peak at three different positions (peak start, peak apex, and peak end). Calibration data for ketamine are shown in Table 1.

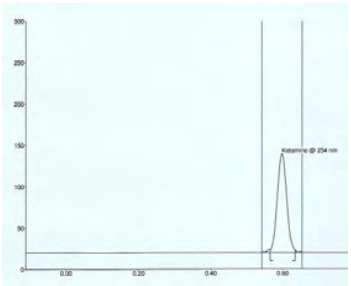

Fig. 2. Densitometric graph of ketamine

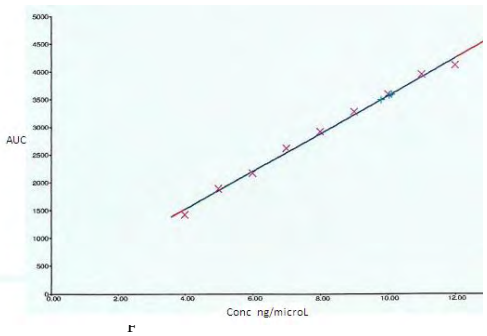

Fig. 3. Calibration curve of ketamine

III. Results and Discussion

A. Selection of Mobile Phase

For the selection of mobile phase, various mobile phase systems have been tried such as toluene:methanol:ammonia (75:25:0.5 v/v); toluene:propanol:ammonia (80:19.5:0.5 v/v) and cycloh exane:toluene:diethylamine (5:3:2 v/v). The system cyclohex ane:toluene:diethylamine (5:3:2 v/v) gave good separation for Ketamine without any interference from excipients. The R_F value for ketamine calculated as 0.61. The UV scanning spectra revealed that at 269 nm it possesses significant absorbance (Fig. 4). So it was selected as detection wavelength.

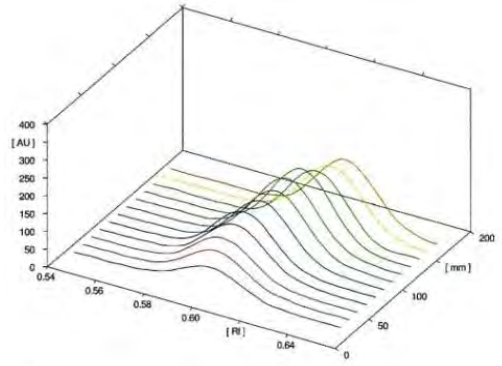

Fig. 4. Densitogram of the calibration

B. Accuracy

To investigate the accuracy in sample preparation (i.e. extraction efficiency), we prepared a spiked solution by adding known amounts of related substances into a sample matrix. There after responses of the spike solutions and the neat standard solutions were taken to assess the recovery from the sample preparation. Recovery studies were carried out by addition of standard drug to the sample at three different concentration levels (i.e. 50%, 100%, and 150%), taking into consideration percentage purity of added bulk drug samples. The method was found to be accurate with % recovery 98.92 - 100.26% for ketamine (Table 2). The formulation obtained from open market was also analyzed by the same method. The assay result was found to be 99.73 ± 0.31% of marketed formulation.

Table I: Regression Analysis Data of Calibration Curves

Conc (ng/spot)	*Area mean ± SD (n = 3)*	*% RSD**	*Conc (ng/spot)*	*Area mean ± SD (n = 3)*	*% RSD**
400	1407.23, 4.35	0.31	900	3265.00, 5.99	0.18
500	1868.84, 4.822	0.26	1000	3590.33, 4.29	0.12
600	2158.17, 9.52	0.44	1100	3938.22, 4.317	0.11
700	2607.47, 7.30	0.28	1200	4106.17, 3.08	0.8
800	2907.05, 4.53	0.16			

*Coefficient of Variation or % RSD

Table II: Results of Recovery Study of Ketamine

Amount of sample Ketamine, ng/spot	*Amount of added Ketamine, ng/spot*	*Amount of recovered Ketamine, ng/spot*	*% Recovery*
1000	0	-	-
1000	1000	995.42	99.5
1000	1500	1510.12	100.7
1000	2000	2010.12	100.5

C. Precision

Precision was calculated as repeatability and intra-day and inter- day variation for ketamine. The method was found to be precise with CV 0.43 - 0.67 for intra-day (n = 3) and CV 0.58 - 1.45 for inter-day (n = 3) (Table 3).

Table III: Precision for Ketamine

Conc. Ng/spot	*Peak Area Intra-day (n = 3)*	*CV*	*Peak Area Inter-day (n = 3)*	*CV*
900	3265.00	0.18	3221.86	0.32.
1000	3590.33	0.11	3565.24	0.16
1100	3938.22	0.11	3964.89	0.21

D. Other Statistical and Validation Parameters

The method was found to be rugged (t-test = 1.13 < t-tabulated = 4.30) with two different HPTLC instrument conditions (different temperatures, 20°C and 30°C). The method was also found to be specific as no interference was observed when the drug was estimated in the presence of excipients. The method was also rugged as there was no change in area up to 48 h of preparation of solution in the mobile phase. Limit of detection (LOD) and limit of quantification (LOQ) of sample were found to be 100 ng/spot and 303 ng/spot, respectively. The summary of validation parameters is tabulated in Table 4. Final optimized conditions are tabulated in Table 5.

Table IV: Summary of Validation Parameters

Parameters	*Ketamine*
Recovery %	99.5-100.7 --
Repeatability (RSD, *n* = 6)	0.46
Intra-day (*n = 3*)	0.11-0.18
Inter-day (*n = 3*)	0.21-0.32
Specificity	Specific
Solvent suitability	Solvent suitable for 60 hr

Table V: Optimized Chromatographic Conditions for Ketamine

S. No.	*Parameter*	*Conditions*
1	Mobile phase	Cyclohexane:toluene: diethylamine (5:3:2 v/v)
2	Stationary phase	Pre-coated silica gel GF60 F (20×10 mm thickness 0.2 mm)
3	Temperature	27°C
4	Distance run (mm)	70
5	Chamber saturation time (min)	20
6	Scanning speed (mm/sec)	20
7	Detection wavelength	269
8	Retention factor (*R*F)	0.61
9	Diluent	Methanol

IV. Conclusions

This method provides a simple, sensitive and precise HPTLC (high performance thin layer chromatography) quantification of ketamine in bulk and pharmaceutical formulations. The separation of ketamine was achieved over HPTLC glass plate

of silica GF-254 by using cyclohexane:toluene:diethylamine (5:3:2 v/v) solvent system. Densitometric evaluation was carried out in the UV region at wavelength of 254 nm. The method was found to be accurate (98.92–100.26%) and precise (CV = 0.43–0.67 for intra-day and CV = 0.58–1.45 for inter-day) within the corresponding linear range. The limit of detection (LOD) and limit of quantification (LOQ) of sample have been found to be 100 ng/spot and 303 ng/spot, respectively.

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