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SEPARATION AND IDENTIFICATION OF ALPRAZOLAM FROM DIACETYLMORPHINE ALONG WITH OTHER OPIUM ALKALOIDS BY HPTLC

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ABSTRACT

Under Narcotic Drug and Psychotropic substances Act 1985, Forensic Science laboratories in Maharashtra state are receiving number of cases. These drug samples are usually not in pure form. Some of the drugs like Analgesics (paracetamol, aspirin), Sedative drugs (phenobarbital, caffeine); Tranquilizers (Diazepam, Lorazepam), Anxiolytic drug (alprazolum), Hypnotic drug (Nitrazepam) etc. Were also used as additives and adulterants to increase quantity and hence for large gain. It is very difficult to detect the diluents and additives used. The reported solvent systems were failed to separate alprazolam from heroin because of interference of opiate alkaloids with alprazolum. Here we have made an attempt to develop glacial acetic acid: water: methanol: ethyl acetate (1:15:20:80) as new, specific, cost effective and reproducible solvent system for HPTLC for the separation of alprazolum, a narcotic drug used as diluents in heroin. The lowest limit of detection for this system was 0.5ppm and specifically useful for separation of alprazolam from heroin along with other opium alkaloids.

KEY WORDS

Additive, Alprazolam, Diacetylmorphine, Solvent system, HPTLC

INTRODUCTION

HPTLC is important analytical separation technique. The main advantage of HPTLC is simultaneous separation of several samples on a single plate needs small amounts of developing solvents can separate milligram amount of sample. Use of automatic sample application device, UV/fluorescent detectors, densitometer scanner (which converts the spot on a layer in to a chromatogram with series of peak) coupled with software enable HPTLC tool and other instruments to enhance sensitivity of analyte detection. [1, 2, 3]

High performance thin layer chromatography (HPTLC) is a versatile technique for the analysis of large number of chemical substances, drugs and dyes. The technique is easily and conveniently used for routine quality control analysis.[4, 5] The term "Narcotic" is derived from the Greek word "Narkotikos", which implies as state of lethargy or sluggishness. [6.] Narcotic drug are those substances which gives relief from pain and induce sleep but not acceptable socially. Narcotics and psychotropic substances are of natural, semi synthetic and synthetic. On ingestion in body they act on central nervous system and produce an altered mind state and also produce physical and psychological dependencies on the drug.[7]

Identification of adulterated samples referred to forensic laboratories, the psychotropic substances are

detected and identified by spraying Dragondroff's reagent on HPTLC plate which shows orange colored spots with different R_f. The drugs can be detected and identified by their different R_f values.

Varshney, et al [9] described the stability of heroin in methanol. Klemenc, et al [10] found Noscaoine as an adulterant in heroine. Krishnamurthy et al [11, 12, 13] described simultaneous detection of adulterants and coextractants in illicit heroin, profiling of street narcotic by high performance liquid chromatography and caffeine and paracetamol as the main adulterants in heroin and in brown sugar respectively. , Koski, A et al [14] reported that Postmortem forensic toxicology frequently finds alcohol both alone and in combination with drugs. Although benzodiazepines are generally considered as safe but are dangerous with alcohol. But none the author could develop successful method for separation of alprazolam in heroin samples.

INSTRUMENTATION

The HPTLC system (DESEGA) consist of AS30 sample applicator cm equipped with 10 µl syringe, zero grade nitrogen gas cylinder and TLC scanner (Densitometer CD 60 with Deuterium lamp) was operated using software 'ProQunt' located on a personal computer.

HPTLC applicator Model No.: AS-30 Make: DESAGA SARSTEDT GROUP

Plate reader: CD 60

Experimental: Pre coated HPTLC plates were made of silica gel $^{60}F_{254}$ of size 20cm x 20cm MERCK make ware used. All solvents used were of analytical grade purchased from MERCK.

Chemicals and Reagents: The chemicals methanols, ethyl acetate, glacial acetic acid, bismuthsubnitrate, potassium iodide, iodine, alcohol, hydrochloric acid used were of analytical grade. Control samples of heroin, alprazolam were received from drug control laboratory Ghaziabad.

Preparation of Standard Solution: Standard solutions (1mg/ml) of heroin, alprazolam, morphine and caffeine were prepared in methanol. Standard solutions of 5ppm, 7ppm, 10 ppm, 12ppm and 15ppm of heroin and alprazolam were obtained from from1mg/ml standard stock solution in methanol.

Preparation of suspected sample Solution: Suspected sample solution of 10mg/ml prepared in methanol.

Chromatography: Standard solutions of alprazolam, heroin and caffeine (2 µl of 1mg/ml) in methanol was applied on precoated silica gel $^{60}F_{254}$ (20x20 cm) glass plate with the help of applicator (AS-30) using spot length 3mm. Suspected sample solution (10mg/ml) prepared in methanol were also applied on same plate with spot length 3mm. The developed solvent system glacial acetic acid: water: methanol: ethyl acetate (1:15:20:80) was used for development of plate up to 10 cm distance. The developed plate was dried in air and then sprayed with alcoholic iodine followed by alcoholic HCl and Dragondroff's reagent (**Fig.1**).

RESULTS AND DISCUSSIONS

Generally samples received in forensic science laboratories are not in pure form and many times are in trace quantities, make analysis very critical. Also many diluents are present in these samples and become difficult to identify all the drugs present in the samples. The solvent systems like TA and TB, do not give separation between Alprazolam, caffine & morphine. Similarly, TC, TD, TE, TF, TL, TAD. TAE, TAF, either heroin or some opium alkaloid interferes with alprazolam. Therefore, it is tedious job to separate alprazolam from heroin along with other opium alkaloid. [15]

The solvent system glacial acetic acid: water: methanol: ethyl acetate (1:15:20:80) give very good separation of these drugs. Even the separation spots can be visualization instead of UV. We have done it by spraying Dragondroff's reagent for specificity. The found Rf values of Alprazolam 0.92; heroin 0.45; Monodactyl morphine 0.30, Caffeine 0.80 and Morphine 0.02 itself indicated good separation. The R_f values of suspected samples matches with that of standard values of alprazolam, heroin monodactyl morphine, caffeine and morphine (**Fig. 1**).

Standard solution of 5ppm, 7ppm, 10 ppm, 12ppm and 15ppm of heroin were made from1mg/ml of heroin standard stock solution in methanol and spotted on HPTLC plate. Spotted plate run in mobile phase glacial acetic acid: water: methanol: ethyl acetate (1:15:20:80) eluent. Table 1 showed area of

standard heroin sample. From the table area of heroin standard verses concentration in ppm plotted. As shown in graph no1 the graph is straight line with R^2 0.996 indicates linearity ion measurements.

Standard solution of 5ppm, 7ppm, 10 ppm, 12ppm and 15ppm of alprazolam were made from1mg/ml of alprazolam standard stock solution in methanol and spotted on HPTLC plate. Spotted plate run in mobile phase Glacial Acetic acid: water: methanol: ethyl acetate (1:15:20:80) as a solvent system.

CONCLUSION

New solvent system glacial acetic acid: water: methanol: ethyl acetate (1:15:20:80) was found to be useful for the separation and identification of alprazolam from diacetylmorphine along with other opium alkaloids by HPTLC.

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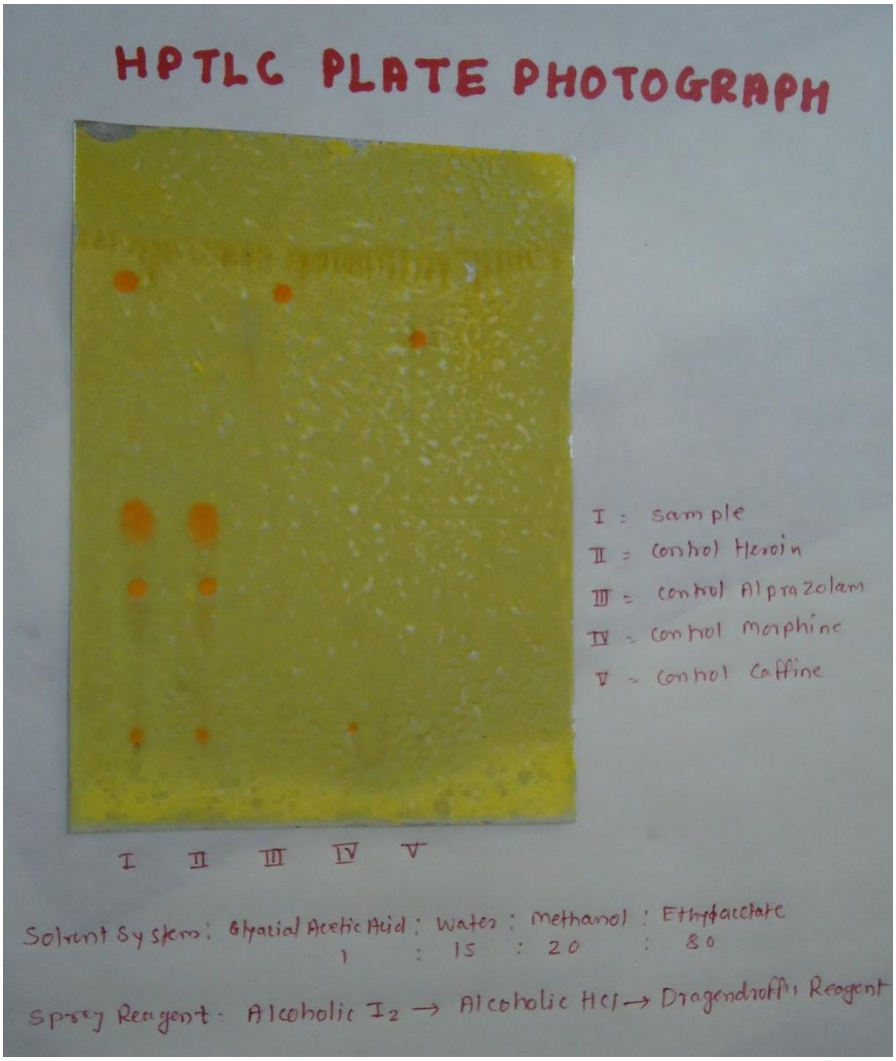
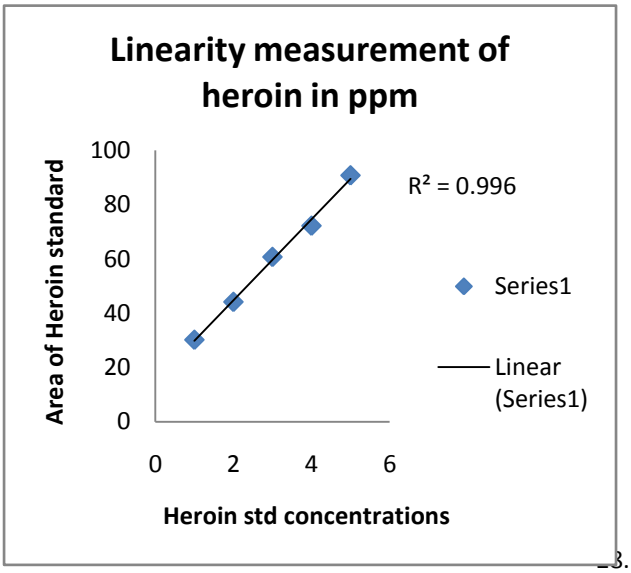


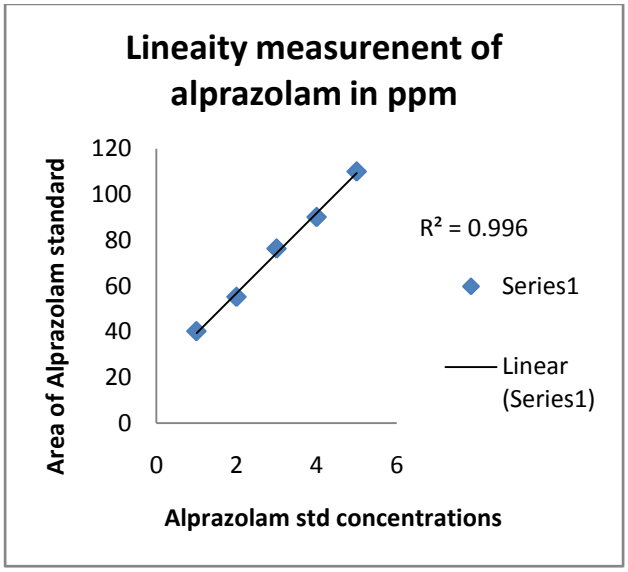
Figure 1: Hptlc photograph showing Seperation of Alprazolam and Heroin

Table 1: Linearity of measurements of hptlc Alprazolam and Heroin samples

Sr.No.	Alprazolam And Heroin Std. Conc.	Area of Heroin standard	Area of Alprazolam standard
1	5ppm	30.21	40.18
2	7ppm	44.18	55.21
3	10ppm	60.76	76.37
4	12ppm	72.25	90.18
5	15ppm	90.83	110.12



Graph 1. Area of Heroin std Vs concentrations in ppm



Graph 2. Area of Alprazolam std Vs concentrations in ppm

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