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Detection and Identification of Dicyclomine in Autopsy Material

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Abstract- In this study, autopsy tissues were extracted and cleaned then subjected to TLC using suitable solvent system. A gas chromatographic-mass spectrometric (GC-MS) method is described for the determination of Dicyclomine residue in autopsy tissue. This method allows detection of residual drug in biological tissues by using single-ion monitoring; confirmation by a full scan electron impact (EI) mass spectrum is possible.

The aim of the paper is to use the proposed technique in cases of drug trafficking, illicit drug seizures and as a test for identity in pharmaceutical and forensic toxicological analysis. The focus of present study has been on methods for detection & confirmation of Dicyclomine in autopsy tissues using GC - MS.

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I. Introduction

Dicyclomine drug is available in the tablet dosage form in the market. It is, also known as Dicycloverine, is chemically 2-(diethyl amino) ethyl-bi (cyclohexane)-1- carboxylate.[1,2] Dicyclomine is used to treat intestinal hyper motility, the symptoms of Irritable Bowel Syndrome (IBS) (also known as spastic colon). It relieves muscle spasms and cramping in the gastrointestinal tract by blocking the activity of acetylcholine on cholinergic (or muscarinic) receptors on the surface of muscle cells. It is a smooth muscle relaxant and it has 72 % of the antimuscarinic power of atropine.[3]

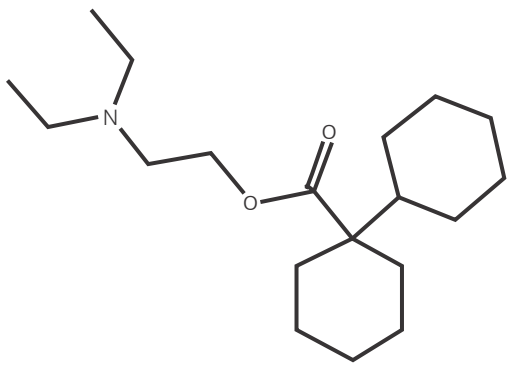

Fig. 1: Chemical structure of Dicyclomine

Literature survey revealed, no article related to TLC and GC MS determination of Dicyclomine in autopsy tissues has been reported. The objective of the present work was to develop an accurate, specific and reproducible method for the determination of Dicyclomine in autopsy tissue s by TLC and GC MS.

An 18-year-old lady found dead in the hostel room of college in Gwalior. The Investigative officer collected tablets from the hostel room and autopsy tissue from post mortem house. All the seized articles were forwarded to the regional forensic science laboratory Gwalior for chemical examination.

The aim of the paper is to use the proposed technique in cases of drug reaction, illicit drug seizures and as a test for identity in pharmaceutical and forensic toxicological analysis. The focus of present study has been on methods for detection & identification of Dicyclomine in autopsy tissues using TLC and GC- MS.

II. Experimental

Standard reagents, Toluene, Acetone, Methanol, Ammonia, Potassium thiocynate, Cobalt chloride and Sodium acetate used were AR grade. Dicyclomine pure, doubly distilled water was used throughout the study.

a) Preparation of Standard Stock Solutions

Standard stock solution of concentration 1000 µg/mL for Dicyclomine were prepared using methanol. From the standard stock solution, the mixed standard solutions were prepared using methanol to contain 100 µg/mL of Dicyclomine. The stock solution was stored at 2-8 °C protected from light.

b) Preparation of Chromogenic Reagent

Potassium thiocyanate (6.06 g), Cobalt chloride (5 g) and Sodium acetate (3.4 g) were dissolved in sufficient water, 2.5 mL of 1 N HCl was added and volume was made up to 25 mL with water. From this solution 20 mL was further diluted to 50 mL with methanol, filtered and stored at room temperature.[4]

Samples: Dicyclomine tablets and autopsy tissue.

c) Extraction of Dicyclomine from Autopsy Tissue and Cleanup of Extracts

In a portion of about 100 g of autopsy tissues (stomach, intestine, lung, liver, spleen and kidney) containing the Dicyclomine drug, 10g Ammonium sulphate was added and minced. Then biological sample was made alkaline with the help of ammonia and extracted with methanol. The filtrate was evaporated. The extracts were subjected to clean up by

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passing through the mixture of silica gel G and activated charcoal filled column having glass wool at the bottom. Finally the collected filtrate was evaporated over hot water bath and used for identification of Dicyclomine.

d) Thin Layer Chromatographic Analysis[5]

Aliquots of standard Dicyclomine and extract obtained were spotted on to the plate, which was developed with toluene: acetone: methanol: ammonia in the ratio of (7: 1.5: 1: 0.1) (v/v/v/v); it gave spot of Dicyclomine at Rf value 0.76 ± 0.02, in a pre saturated TLC chamber, to a height of 10 cm. The plate was removed from the chamber dried in air and sprayed with chromogenic reagent, which forms a blue-colored spots against light pink background.

The Rf value of Dicyclomine can be compared with the obtained spots of extract.

e) TLC Method Optimization and Chromatographic Conditions

The TLC procedure was optimized for estimation of Dicyclomine. The standard stock solution 100 µg/mL of Dicyclomine) were taken and 10 µL samples were spotted on to TLC plates and run in different solvent systems. Initially, toluene, acetone and methanol were tried in different ratios but perfect spots were not obtained. Hence, ammonia was tried along with above mobile phase. Finally for effective separation of Dicyclomine, the mobile phase containing a mixture of toluene: acetone: methanol: ammonia (7: 1.5: 1: 0.1v/v/v/v) was found to be optimum. The above mobile phase improved the spot shape and gave suitable Rf value for Dicyclomine. In order to reduce the neck less effect TLC chamber was saturated for 30 min. The plates were developed for a distance of 80 mm and then dried in hot air, which takes approximately 20 min for complete development of the TLC plate. As Dicyclomine is non UV absorbing compound, it could not be scanned under UV detector. After the TLC plate was developed in mobile phase, derivatizing agent was poured on the plate and dried. Blue spots against light pink background were developed within 20 min as later background starts getting darker.

f) Gas Chromatograph Mass Spectrometer

GC-MS studies were performed on Agilent technologies 5973 inert model mass selective detector using Column HPSMS 0.25 mm id 30 m length, 0.25µ film thickness, 30mx250µmx0.25µm nominal with aux temp 280°, intel temp 250° MS quadrupole 150 ion source 230, column flow 1.0 ml/min He as carrier gas, split mode 20:1 programming 100°C 2 minute hold 20°C/min ramp up to 280°, 500 volt total 16 minute run.

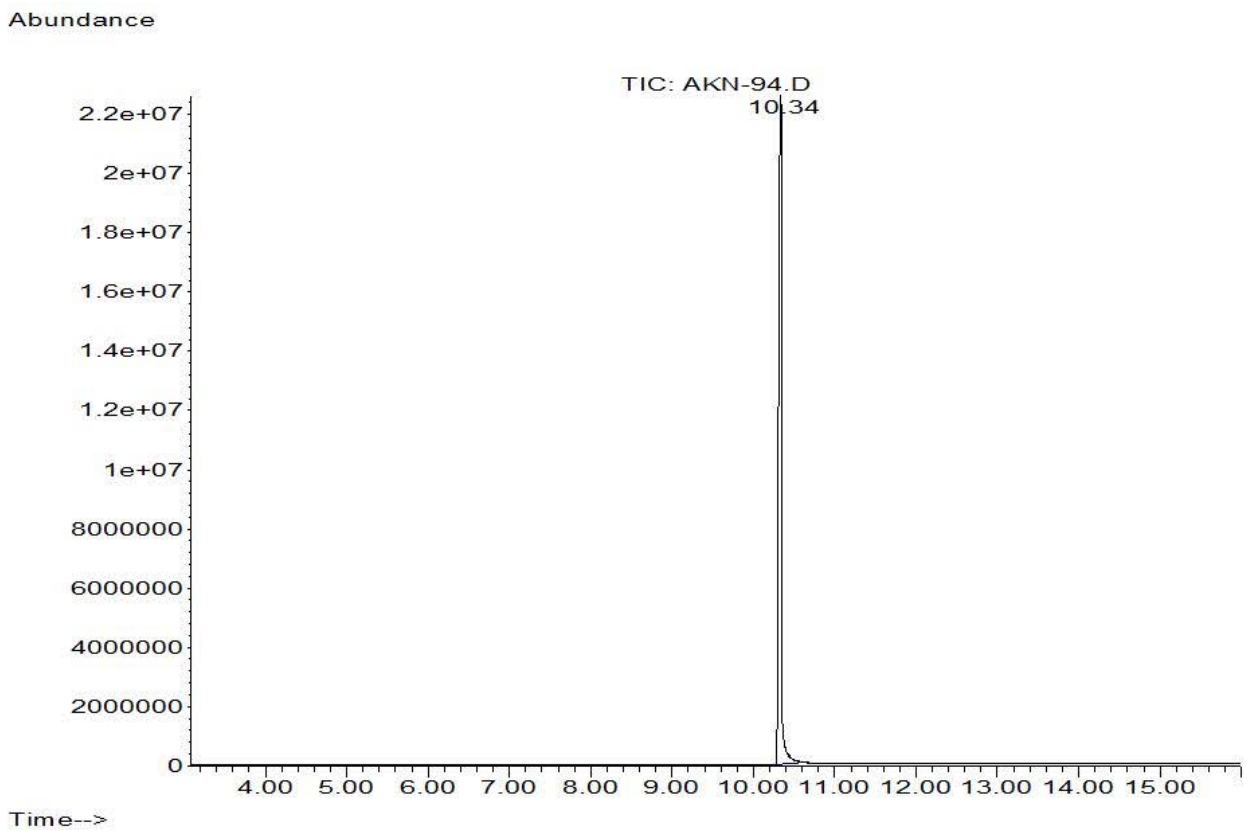

Fig. 1: Total Ion Chromatogram of Extract from Exhibits

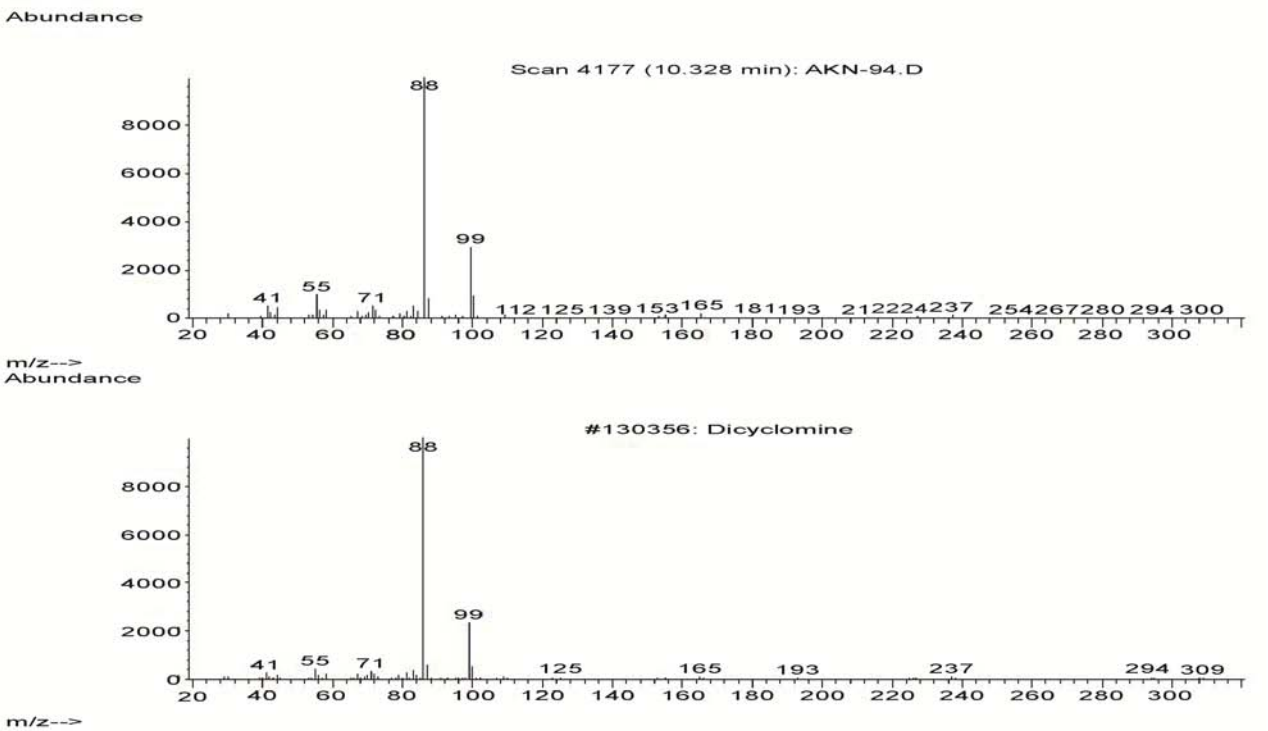

Fig. 2: Mass Spectrum of Extract and their Library Match

III. Results and Discussion

Dicyclomine can be successfully detected on TLC plates with good sensitivity.

The extract was injected into the GC-MS apparatus, and a total ion chromatogram and a full scan mass spectrum were obtained. Figure-1 shows the TIC and Figure-2 shows the mass spectrum of Dicyclomine with their library match, in this work; we attempted to detect Dicyclomine using selected ion monitoring.

The detection limit was determined by analyzing samples at various concentrations with this method, and it was found that 0.01 μg/ml of residual Dicyclomine in a sample can be detected using SIM. Figure 1 shows the total ion chromatogram at equal sensitivity of extracts from blank samples. The peak of Dicyclomine can be clearly identified, thus the detection limit was determined to be at least 0.01 μg/ml in sample. However, many background ions appeared on the full scan mass spectrum of this peak.

IV. Conclusion

Today, TLC is rapidly becoming a routine analytical technique due to its advantages of low operating costs, high sample throughput, and the need for minimum sample preparation. The major advantage of TLC is that several samples can be run simultaneously using a small quantity of mobile phase-unlike HPLC; thus reducing the analysis time and cost per analysis. The developed TLC technique is precise, specific, and accurate. Statistical analysis proves that the method is suitable for the analysis of Dicyclomine as a bulk drug and in pharmaceutical formulation without any interference from the excipients. It may be extended to study the degradation kinetics of Dicyclomine and also for its estimation in plasma and other biological fluids.

The method is simple, fast and reliable with no interference from common drugs. The method developed and the analysis of Dicyclomine in tissues could prove that the Dicyclomine were taken by deceased and the subsequent reaction had caused the death.

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