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Detection & Identification of Paracetamol from biological material using HPTLC combined with Modern analytical technique

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ABSTRACT:

A simple rapid and accurate High performance thin layer chromatography (HPTLC) method has been developed for identification of paracetamol from biological material, i.e. blood, gastric larvage, and visceral material. The method is based on HPTLC separation of paracetamol-analgestic drug followed by densitometric measurements of spt at 270 nm. The separation was carried out on HPTLC plate using Benzene: ethanol (90:10) as mobile phase. The method was developed with new chromogenic spray reagent formaldehyde-sulfuric acid. Paracetamol gives dark green coloured spot at respective RF values with above solvent. Other organophosphorus, organochlorine, pyrethroid, and carbamate insecticides and constituents of viscera (amino acids, proteins, peptides, etc.) do not react with this reagent. The detection limit is ca 10 μg.

Key Words: Forensic science**,** HPTLC**,**GC**,**GC-Ms**,** Densitometry Paracetamol

INTRODUCTION:

Paracetamol (N-(4-hyolledroxyphenyl) acetamide) is usually simplyAbbreviated as APAP, for N-acetyl-para-aminophenol, often more commonly known by its alternative name acetaminophen. It's widely used for management of pain and fever in a variety of patients including children, pregnant women and the elderly[1]. Paracetamol, is often the analgesic or

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antipyretic of choice in patients in whom salicylates or other NSAIDs are contra-indicated. Such patients include asthmatics or those with a history of peptic ulcer, or children in whom salicylates are contra-indicated because of the risk of Reye's syndrome[2].

History:

Paracetamol is part of the class of drugs known as aniline analgesics, it is the only such drug still in use today[3]. The first observations about the analgesic and antipyretic properties of paracetamol were made back in the late nineteenth century when alternative compounds were being sought to reduce fever in the treatment of infections. The antipyretics commonly used at the time consisted of preparations of natural compounds such as cinchona bark, from which quinine is derived, or galenicals based on willow bark, the earliest source of salicylate. Cinchona bark became in short supply and cheaper synthetic substitutes were needed. Two alternatives that were developed included acetanilide in 1886 and phenacetin in 1887, both of which had the advantage over quinine of possessing both antipyretic and analgesic properties. In 1893, another compound, now known as paracetamol, was noted also to have a prompt analgesic and antipyretic action[4]. Paracetamol was first marketed in the United States in 1953 by Sterling-Winthrop Co., which promoted it as preferable antipyretic to aspirin since it was safe to take for children and people with ulcers. In 1963, paracetamol was added to the British Pharmacopoeia, and has gained popularity since then as an analgesic agent with few side-effects and little interaction with other pharmaceutical agents[3].

Physical Properties:

Paracetamol is a white or almost white, crystalline powder. Sparingly soluble in water, freely soluble in alcohol, very slightly soluble in methylene chloride[5]. Paracetamol consists of a benzene ring core, substituted by one hydroxyl group and the nitrogen atom of an amide group in the para (1, 4) pattern. The amide group is acetamide (Ethanamide). It is an extensively conjugated system, as the lone pair on the hydroxyl oxygen, the benzene pi cloud, the nitrogen lone pair, the p orbital on the carbonyl carbon, and the lone pair on the carbonyl oxygen is all conjugated. The conjugation also greatly reduces the basicity of the oxygen and the nitrogen,

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while making the hydroxyl acidic through delocalization of charge developed on the phenoxide anion[6].

Toxicity :

The toxic dose of paracetamol is highly variable. In adults, single doses above 10 grams or 200 mg/kg of bodyweight, whichever is lower, have a reasonable likelihood of causing toxicity[7]. Acetaminophen toxicity may result from a single toxic dose, from repeated ingestion of large doses of acetaminophen (e.g. 7.5-10 g daily for 1-2 days), or from chronic ingestion of the drug. Dose-dependent, hepatic necrosis is the most serious acute toxic Effect associated with over dosage and is potentially fatal[8]. Acute overdose of paracetamol can cause potentially fatal liver damage. A normal dose can be hepatotoxic by alcohol consumption. Paracetamol toxicity is the foremost cause of acute liver failure in the Western world and accounts for most drug overdoses in India, the United States, the United Kingdom, Australia and New Zealand[9-10].

Case History:

In our laboratory one case of poisoning was received from police authority , seven year old child was suffering from acute liver failure as result of paracetamol poisoning. The child died during hospitalization. Viscera along with blood sample were forwarded to our laboratory for exact cause of death. Now days despite paracetamol poisoning being one of the common poisoning in our country.

Literature survey:

Literature survey reveals there are UV, HPTLC, HPLC methods reported for detection and identification of paracetamol. Various reagents are reported in literature for the detection and determination of Paracetamol by TLC and HPTLC. A sensitive spray reagent of ferric chloride is a routine reagent[11]. HPLC is the most modern method used for detection and identification of paracetamol from biological material but it is very expensive and requires highly qualified instruments. A simple TLC method was effectively used for the detection of paracetamol. Toluene: acetone: acetic acid (20 ml: 20ml: 20 drops) was chosen as a preferable mobile phase for this purpose. We choose this mobile phase after trials of several mobile phases and it was the best one that gives a good spot separation and better RF values.

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Present work:

We developed a new chromogenic spray reagent for chromatographic detection and identification of paracetamol. Formaldehyde – sulphuric acid and heat is applied for HPTLC gives green color complex. Actually Formaldehyde – Sulfuric acid reagent is used for detection of Petroleum hydrocarbon[12].

Material and Method:

All reagents and Chemicals used were of analytical reagent grade. Glass distilled water was used throughout.

Standard solution of paracetamol:

1 g of STD paracetamol powder was weighted accurately in 50 ml of methanol.

Formaldehyde-Sulphuric Reagent:

3ml of 10% Formaldehyde is added in 5 % sulphuric acid

For Extraction procedure:

2 ml of phosphate buffer (ph 7.4)+40 ml of Chloroform+ 4 ml of blood + 2 gm unhydrous Sodium Sulphate ,decant Chloroform filter the solution and concentrate it for 10 min.

Apparatus:

HPTLC Condition: Chromatography was performed on 10 cm × 10 cm Silica gel F_{254} HPTLC plate (Merck, Darmstadt, Germany #1.05729 OB397077) a Desaga (Heidelberg, Germany) AS 30 TLC applicator, spotting volume 5 ml, spotting rate 10 s ml^{-1} was used to apply standard stock solution of Paracetamol (1mg/ml^{-1}l) and other standard solution of organophosphorous insecticide such as dimethoate, phosphomidon, dichlorovas, Malathion, ethyl parathion, methyl parathion, phorate, Organochlorine insecticide such as endosulfan, DDT and BHC, Carbamate insecticide such as Propoxur(baygon), Carbaryl, carbofuran, Carbosulfan and Pyrethroid insecticide such as Fenvalrate, Cypermethrin and deltamethrin and other Tranqulizer,spotted along with extracted solution (5μl stock sample solution). The HPTLC plate was then developed in a previously saturated HPTLC chamber using various solvent systems. Saturate the chamber ½ hour before developing the HPTLC Plate. When the solvent had

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migrated to 10 cm, the plate was removed and allowed to dry at room temperature for 10 minute and sprayed with 10% Formaldehyde-sulphuric acid heat the plate for 10 min green color spots were observed at R_F 0.6 for standard Paracetamol and blood extracted solution.

1) Densitometry: The sample and STD paracetamol was spotted as described above paracetamol was spotted as described above. Densitometric scanning was performed on AS 30 TLC scanner at 230 nm. The source of radiation utilized was Deuterium lamp. Evaluation was performed using linear regression analysis via peak areas

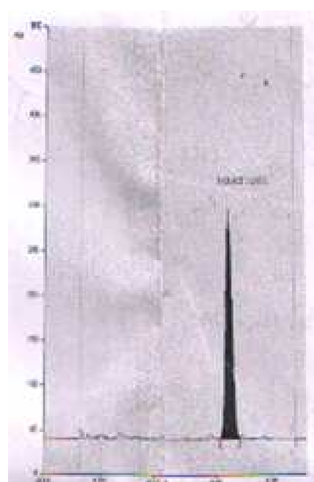

Fig 1: Typical densitogram of sample

2) UV-Spectrophotometer (Specord S 100)

UV range 200-400 nm, Medium: 0.1 N HCL Threshold-10

Blood sample was analyzed and Max was noted

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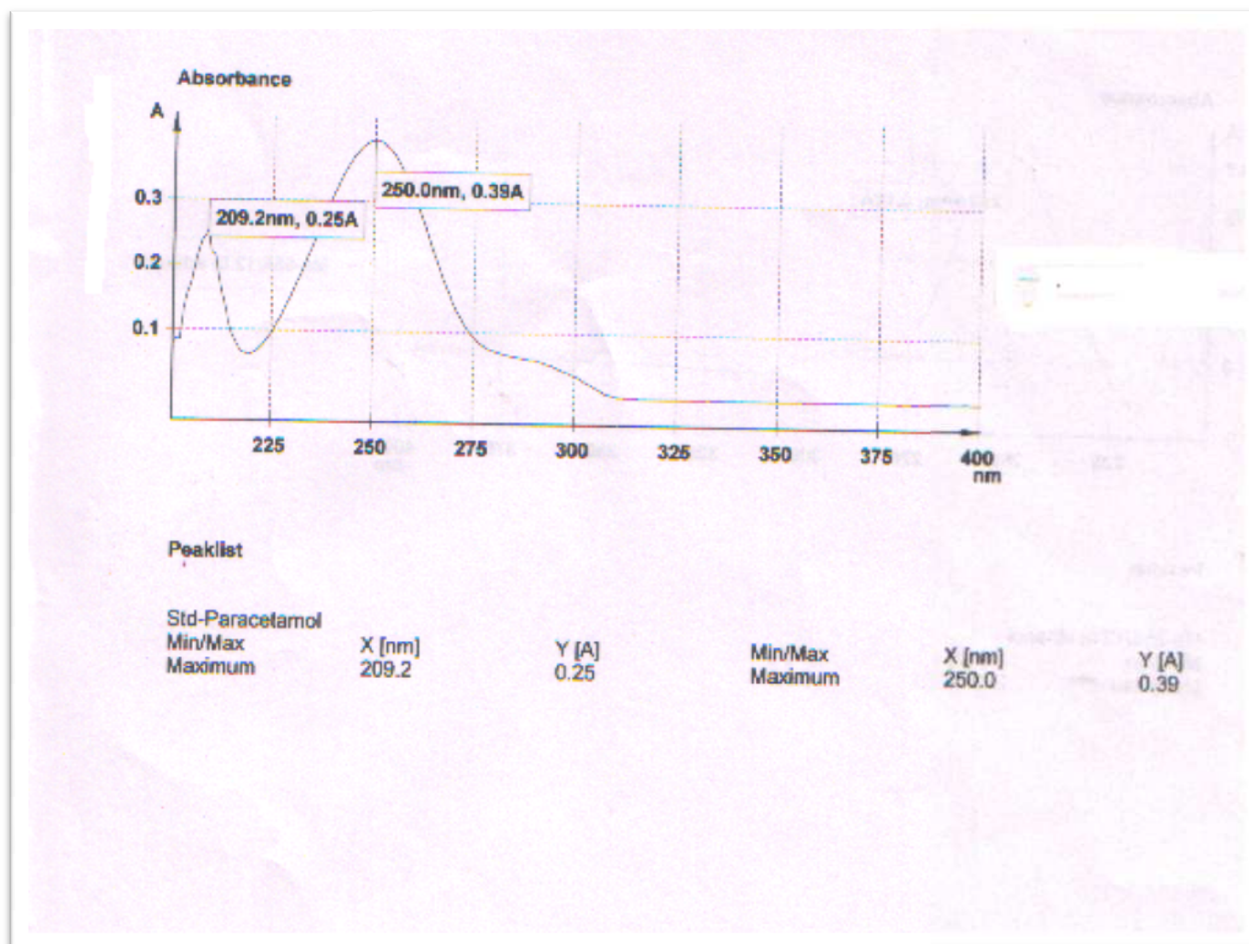

3) LC-MS(Bruker)”

Methanol extract of the sample was subjected to the LC-MS analysis. The chromatograph indicated one distinct spot at Rf 0.6. The preparative TLC was done and the methanol extract of eluted spot was injected to LCMS. For identification, The peak shows presence of paracetamol (M+1) at 152

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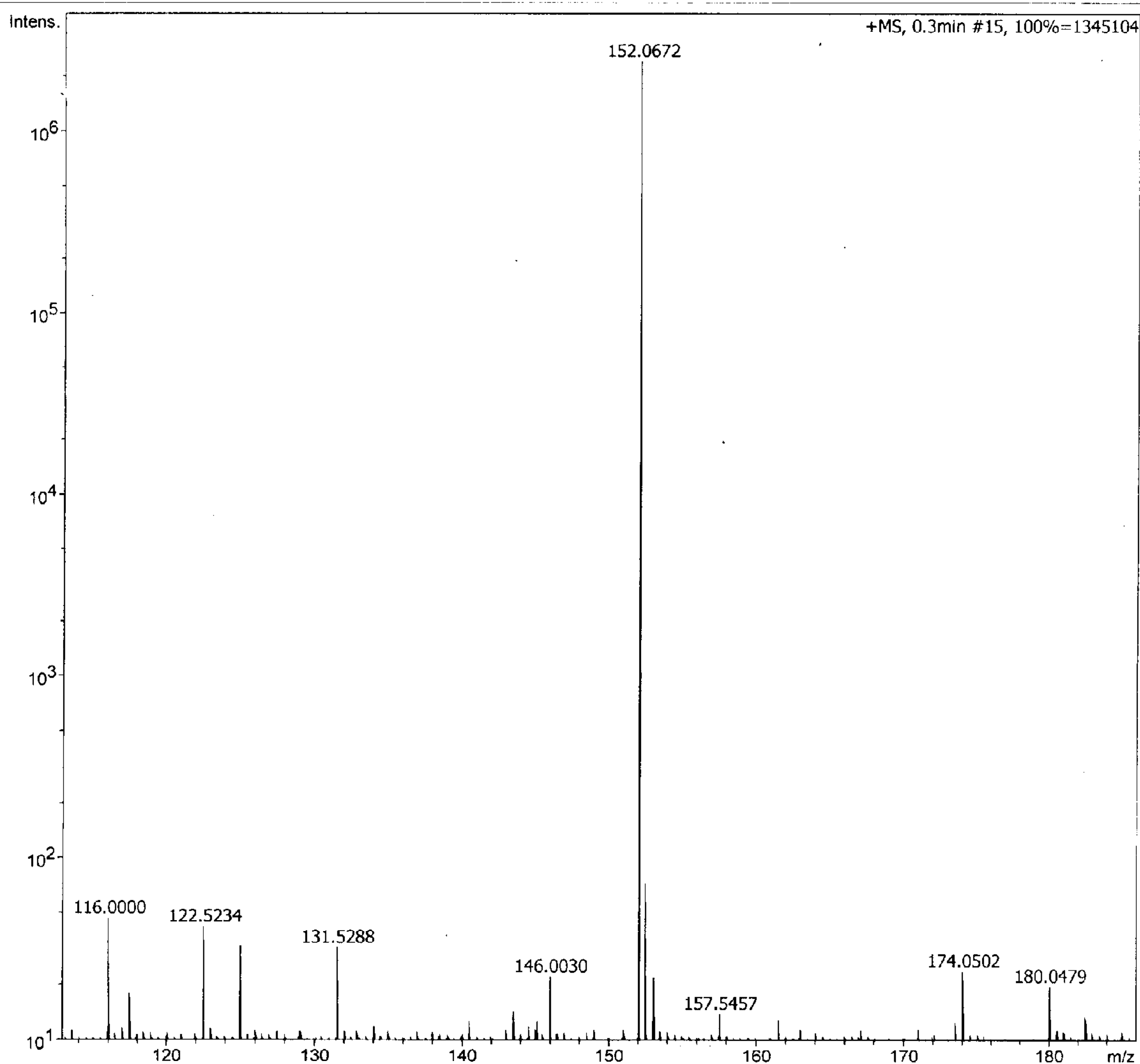

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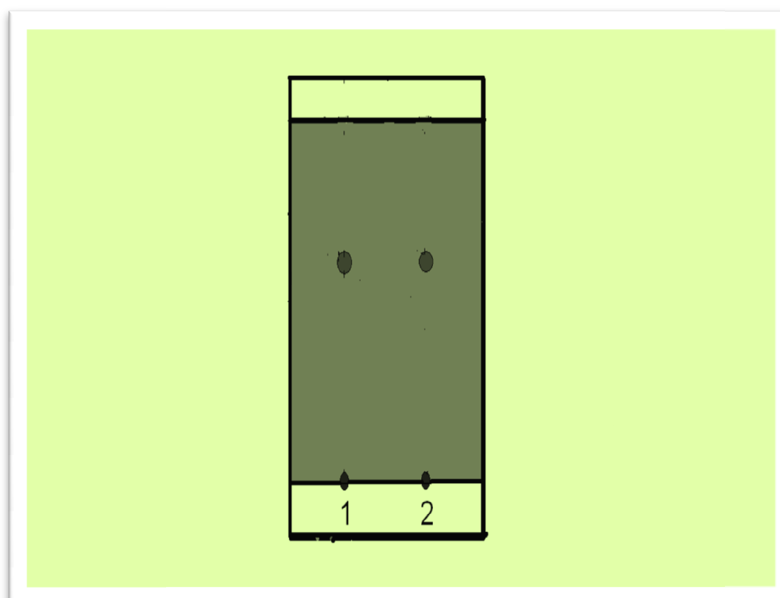
Determination of Recovery

1 mg of sample of Paracetamol in methanol was added to 10 ml. of normal fresh blood, mixed well and kept for a day. The blood sample was then extracted with buffer as described under 'Extraction'. The extract was evaporated to dryness and the residue was dissolved in to 1 ml of methanol. A 5µl volume of this solution was spotted on HPTLC Plate together with 5µl each of standard technical paracetamol solution containing known concentration, 0.5, 0.75, 1, 1.25, 1.5, 2, 2.5, 3 mg per 10 ml of methanol. The plate was then developed as described earlier and sprayed with above reagent. Green colour spots were observed at R_f 0.6. The intensity of the spot developed for the visceral extract was visually comparable with the spot corresponding to 0.75 mg per 10 ml (average of three experiments); hence the recovery was Calculated as 90%.



Results and Discussion:

Drug detection i.e. Identification, Detection, Quantization of drugs from biological Method is one of the most important factors in Toxicology section. Many times biological sample received in Forensic Laboratories are not in proper condition. Therefore, analysis of this sample is difficult.

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From the chromatograph it is clear that hR_f of paracetamol matched with hR_f of blood sample. Different mobile phases A, B, C and D were tested to get good detection of paracetamol. The best mobile phase for identifying paracetamol is Toluene : acetone : Acetic acid.

The chromatograph indicated one distinct spot at R_f 0.6. The preparative TLC was done and the methanol extract of eluted spot was injected to LCMS. For identification, The peak shows presence of paracetamol (M+1) at 152. This spray reagent gives positive reaction for petroleum hydrocarbon, but giving different color and different R_f .Value. This spray reagent gives specific color and specific Rf. For paracetamol from biological material. UV-Spectrum and Densitogram is matched with standard. So this is the simple, rapid, sensitive method for detection of paracetamol from biological material.

	Mobile Phase	**R_f**
a	Toluene : Acetone : Acetic Acid (20:20:1)	0.60
b	Benzene : Methanol (90:10)	0.35
c	Butanone	0.44
d	Chloroform : Methanol (10:10)	0.46

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