Short Communication

Detection of Paracetamol in Skin Tissue by GC-MS - A Case Study

Jain RK, Jayshanker G, Sarin RK*

ABSTRACT

A 13 year-old child suffered an anaphylactic reaction to a drug combination and died in the clinic. The Investigating Officer collected an assortment of drugs and injection vials from the clinic and forwarded them along with the injection site of the victim's skin and some viscera to the laboratory for examination.

At the laboratory, the samples were subjected to chemical and chromatographic analysis. After extracting the drug from the biological samples, TLC was done using a new solvent system, which improved the separation of drug from the matrix. A gas chromatographic-mass spectrometric (GC-MS) method devised for the determination of paracetamol residue in skin tissues was done. This method allows for detection of residual drug in biological tissues by using single-ion monitoring, and confirmation by a full scan electron impact (EI) mass spectrum. Paracetamol was extracted with ether/chloroform from the samples after acidic and basic extraction procedures, cleaned up and washed, followed by partition between chloroform. The cleaned up extract was injected into the GC-MS, and detection was done using single ion monitoring at m/z 109.

Key words: *GC-MS, paracetamol*

Introduction

Paracetamol (acetaminophen) is generally used as an analgesic-antipyretic for the treatment of bodyaches, fever, etc. It is available in injection and tablet forms. The drug can have direct toxic effects depending on the dose and age of the patient.[1] Some individuals develop a severe allergic reaction to paracetamol even at very low levels of exposure. This is more common with the parenteral preparations.

In the case of death of the patient, paracetamol can be extracted from postmortem tissues by using modified Stass-Otto/ammonium sulphate procedure with solutions of weak mineral acids, and basic extraction with organic solvents.[2] Methods used to determine paracetamol are mostly based on chromatographic techniques: TLC, GC, and HPLC.[3] These methods however lack specificity, and in addition, TLC lacks sensitivity. A chromatographic GC FID method for paracetamol has also been reported, which too is not very sensitive.[4] Further, several investigators have published methods for determination of paracetamol in body fluids such as plasma and urine, but not in tissues.

The focus of this study has been on modification of existing methods for the detection and confirmation of paracetamol in biological tissue (skin) using GC-MS.

Materials and Methods

Apparatus: GC-MS, TLC

GC-MS: GC Column: Capillary column 5% phenyl methyl silicone, 30 mts , 0.25 mm id, 0.25 mm film thickness.

GC-MS: Perkin Elmer with auto system XL GC, Turbo mass, Auto sampler, 2ml injection, injector port 200, interface 200 degree, EI mode , 70 eV, Mass range 40-630 amu.

Operating conditions: Injection port- 200 degrees, oven programming- 70 degree hold 1 min @ 5 degree to 200 degree hold 1 min and up to 250 degree hold for 5

*(*Corresponding author*) Central Forensic Science Laboratory, Directorate of Forensic Science, Ministry of Home Affairs, Government of India, Ramanthapur, Hyderabad 500013

min; carrier gas helium flow- 1ml/min, ionization voltage: 70 ev.

TLC: A modified solvent system was used, where separation was found to be better.

Solvent system: Chloroform: Acetone: Ammonia (4: 8: 0.2)

hRf was found to be 17.

Spray reagent: Dragon-droff reagent- Orange colour.

Reagents and chemicals: Standard reagents-methanol, n-hexane, chloroform, ammonia solution (all AR grade).

Paracetamol pure; de-ionized double-distilled water was used through out.

TLC plates silica gel F_{254} were obtained from E-Merck (Darmstadt, Germany).

Standard solutions

Stock solution: Accurately weighed 50 mg of pure paracetamol dissolved in methanol solution, made in 5 ml standard flask to a concentration of 10 mg/ml.

Working solution: Dilute standard solutions to 1.0, 5.0, 10.0 mg/ml concentration with methanol from the above stock solution. These solutions were stored under refrigeration.

Samples

Autopsy skin-prick site, blank skin.

Extraction and clean up

4.0 mg of skin tissue was mashed in 10 ml distilled water and taken into 20 ml centrifuge tube. 1 ml of 5% acetic acid solution was added and then digested in water bath for 30 minutes with saturated ammonium sulphate solution. The centrifuge tube was shaken well for 10 minutes. 10 ml of ether was then added into the extract in separating funnel, shaken vigorously for 5 minutes, and set aside to separate. The aqueous phase was drained into another 50 ml separating funnel, made alkaline with 1N ammonia, and extracted with chloroform. The extraction was repeated twice. Portions of ether extract and chloroform extract were combined, evaporated at room temperature, and analyzed.

Evaluation of repeatability, reproducibility, and linearity

The repeatability of the extraction procedure and chromatographic analysis was determined by 5 replicate injections of the standards prepared as described above. The reproducibility of the method was determined by 5 replicate injections. Linearity of the detector response was evaluated by plotting the total peak area versus total concentration, and performing linear least squares regression analysis.

Discussion

Paracetamol is an amphoteric drug with both acidic and amino groups. Its molecular weight is 151.The extract obtained in the manner described was injected into GC-MS apparatus, and and a total ion chromatogram and a full scan mass spectrum were obtained. **Fig 1** shows the TIC (Total Ion Chromatogram) and **Fig 2** shows the Mass Spectrum, in which the base peak of m/z 109 is very prominent and m/z 43, 80 and 151 are observed but relatively weak. Some investigators suggest that simultaneous monitoring of three ions is to be taken as a minimum for identification by selected ion monitoring of GC-MS spectra.[5]

In this work, an attempt was made to detect paracetamol using selected ion monitoring at m/z 109, 151. However, the sensitivity of detection (<1/10) is not very high.

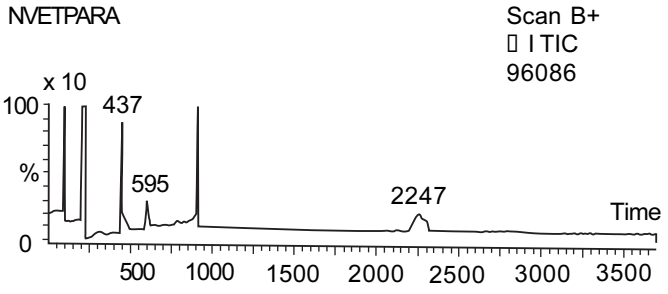

Fig. 1 Total ion chromatogram

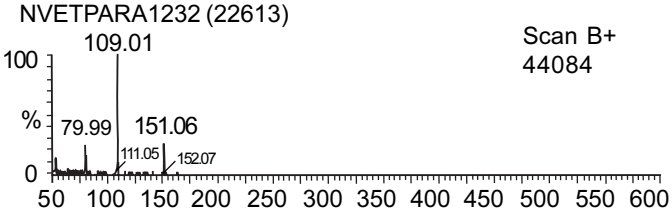

Fig. 2 Mass spectrum

Acknowledgement

The authors are thankful to Dr CN Bhattacharya, Director of CFSL, Hyderabad for providing the facility to carry out this study, and to the scientists of the toxicology division of CFSL Hyderabad for their help and cooperation.

References

1. Moffat AC, editor. Clarke's Isolation and identification of Drugs. 2nd ed. London: The Pharmaceutical Press.
2. Modi NJ. Modi's Medical Jurisprudence and Toxicology. Bombay: Tripathi Private Ltd; 1969.
3. Primus TM, Kohler DJ, Furcolow CA. J Liquid Chromat Related Technol. 2004; 27: 897-909.
4. Moffat AC, editor. Clarke's Isolation and identification of Drugs. 3rd ed. London: The Pharmaceutical Press; 2004.
5. Sphon JA. Use of Mass Spectrometer for confirmation of animal drug residue. J Assoc Analytical Chem. 1978; 61: 1247-1252.