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Research Article

DEVELOPMENT AND VALIDATION OF A GC-FID TECHNIQUE FOR DETECTING METHAMPHETAMINE IN ILLICIT DRUGS SAMPLES IN SRI LANKA

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Abstract

Methamphetamine, commonly known as "ICE" in Sri Lanka, is a potent drug classified within the amphetamine group, functioning as a stimulant for the central nervous system. Data from the Government Analyst's Department (GAD) indicates a notable increase in received methamphetamine cases. As the authorized institute for analyzing and providing reports on illicit drugs to the courts, it is crucial for the Government Analyst's Department to develop and validate a rapid GC-FID method for accurately quantifying methamphetamine. This study involves the qualitative and quantitative analysis of forty samples received by the Government Analyst's Department (GAD) between 2019 and 2021. Presumptive tests, TLC, FTIR, and GCMS methods were primarily used for qualitative analysis, whereas quantitative analysis was carried out using GC-FID. Chromatographic separation was performed on HP-5MS column (30 m x 250 µm x 0.25 µm) with a temperature program of 110°C for 4 min, followed by a ramp up at 10°C/min to 280°C, with nitrogen as the carrier gas at a flow rate of 1.0 ml/min. The method was found to be satisfactory and successfully applied in the quantification of methamphetamine. In the validation of GC-FID method parameters, linearity, precision, LOD, and LOQ were considered. Calibration curves ranged between 50-400 mg/L and 10-50 mg/L, representing correlation coefficients of 0.9978 and 0.9985, respectively. The precision was expressed as Relative Standard Deviation below 2%. The limit of detection and the limit of quantification were 5.32 mg/L and 5.98 mg/L, respectively.

Keywords: Methamphetamine, Qualitative Analysis, GC-MS, GC-FID, Method Validation.

INTRODUCTION

Methamphetamine (MA), a potent and highly addictive stimulant of the central nervous system (CNS), has become a major public health issue with far-reaching implications for both individuals and society. Methamphetamine is an amphetamine-type stimulant (ATS), constituting one of the most significant drug problems worldwide [1]. It stands as the second most widely abused illicit substance globally, following only cannabis in prevalence. The number of MA users exceeds the combined total of heroin and cocaine users. East and Southeast Asia bear the brunt of this issue, hosting approximately two-thirds of the world's MA and amphetamine users. The Americas, particularly the United States and Northern Mexico, account for around one-fifth of the user population [2,3]. The impact of MA is particularly pronounced in several Asian nations such as Brunei, Cambodia, Japan, and Thailand, where it stands out as the primary drug concern. These countries report indicators of abuse, production, and trafficking of MA, reflecting an escalating problem. Additionally, other Asian countries, including China and Vietnam, are witnessing signs of increased MA use, signaling a growing challenge in the region [2]. Sri Lanka, like many other parts of the world, also faces drug-related issues. As a SAARC region country, there is a substantial increase in drug addiction and trafficking over the last two decades in Sri Lanka as situated between the golden crescent and the golden triangle, two major poppy-growing areas, Sri Lanka has become a transit point for illegal drugs entering the country. Trafficking and usage of illicit drugs adversely affect socio-economic policies, safety, and security of the country.

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Sri Lanka faces several social problems, including the growing number of drug addiction, incarceration, and recidivism. In recent years, the most widely used illicit drugs were heroin and cannabis. However, methamphetamine usage has considerably increased according to data from the National Dangerous Drugs Control Board of Sri Lanka [4]. The aim of this study is to develop and validate a GC-FID method for the quantitative determination of methamphetamine in seized drug samples. A GC-FID method was developed and optimized with respect to various parameters including oven temperature, flow rate, and injection conditions. The GC-FID method was validated in terms of precision, linearity, Limit of Detection (LOD), and Limit of Quantification (LOQ).

MATERIALS AND METHODS

Standards and solvents

All the chemicals and solvents used were AnlarR grade. Methanol was obtained from VWR PROLABO chemicals, France. Chloroform was purchased from Sisco research laboratory, India. Conc. Ammonia was purchased from Lobachemie Ltd, India. Cyclohexane, Acetonitrile, Isopropanol, Toluene, and Acetone were obtained from Technophamchem India. Diethylamine and Phenylbenzylamine (PBA) were purchased from Sigma Aldrich, Belgium. The Certified reference Material (CRM) of Methamphetamine was purchased from Lipomed, Switzerland.

Instruments and Equipment

An analytical balance (Mettler, AE 100, Poland) was used for necessary weighing procedures, while Millipore filters (Nylon, 0.45 µm, Agilent Technologies, USA) were used to filter

sample solutions. A digital vortex mixer (VELP, Scientifica) was used during the sample preparation to mix the solutions. SILICA 60 F- 254 plates (20 cm x 20 cm with 0.25 mm film thickness) for Thin Layer Chromatography (TLC) were purchased from Merck, Germany. An FTIR spectrophotometer (Thermo Scientific Nicolet IS10) and GC-MS (Agilent Technologies 7890 N gas chromatograph with 5975C mass spectrometer) were used for qualitative analysis. Gas chromatography with flame ionization detector (Thermo Scientific TRACE 1300) was utilized for quantitative analysis.

Sampling

Forty samples analyzed were obtained by the narcotics section of the Government Analyst's Department between 2019 to 2021. The sample was powdered using a mortar and pestle. The corning and quartering method was employed for sampling to analyze powdered methamphetamine. Initially, the powdered methamphetamine was placed on a flat surface, forming a conical heap, before being spread out into a circular shape resembling a flat cake. Subsequently, the cake was divided into quarters, and two opposite quarters were combined. This procedure was repeated until the sample size became small enough. Powder from each sample was subjected to presumptive tests, TLC, GC-MS, FTIR and GC-FID.

Presumptive tests

Presumptive Tests for methamphetamine were performed using the Marquis test and the Simon's test.

Simon's Test

Aqueous Sodium Carbonate solution (2%), 1% aqueous Sodium Nitroprusside solution, and 50% (v/v) Ethanolic acetaldehyde solution were prepared for the Simon's test. A small amount of powder was placed in a depression on a spot plate. One drop of aqueous Sodium Carbonate was added and stirred. Next, one drop of aqueous Sodium Nitroprusside solution was introduced followed by the addition of one drop of Ethanolic acetaldehyde solution. The resulting color change of the mixture was observed [5].

Marquis test

A small amount of powder was placed in a depression on a spot plate. One drop of 37% (v/v) formaldehyde solution was added and stirred. A drop of concentrated sulfuric acid was added, and the color change of the mixture was observed [6].

Thin Layer Chromatography (TLC)

TLC for the samples, alongside primary reference samples of methamphetamine, was conducted using a mixture of Cyclohexane, Toluene and Diethylamine in a 75:15:10 (v/v/v) ratio. The samples were visualized under the UV light and subsequently sprayed with 2% Ninhydrin (in ethanol). Retardation factors were calculated to confirm the presence of methamphetamine in the samples.

Gas Chromatography-Mass Spectroscopy (GC-MS)

Agilent technologies 7890 N gas chromatograph with 5975C mass spectrometer was used for confirmation of methamphetamine in the sample. HP-5 MS (5% phenyl methyl siloxane) column with dimensions 30 m x 0.250 mm x 0.25 µm was used. Carrier gas was Helium with the flow rate of 0.6 ml/min. Splitless injection mode was used for the injection volume of 1.0 µL. Injector temperature was set to 280°C and the oven temperature program was set starting from 90°C for 2 minutes, then increased with a ramp of 14°C./ min up to 300°C and held for 10 minutes. The total run time was 27 minutes. Unknown samples were injected and analyzed using the Retention time in TIC and the mass spectrum patterns obtained. Data analysis was done using the Agilent MSD Chemstation software.

Fourier Transform Infrared Spectroscopy (FTIR)

Presence of methamphetamine was confirmed using the Thermo Scientific Nicolet IS10 FTIR

Gas chromatography with flame ionization detector

GC-FID analysis was performed to generate standard and samples chromatographs using Thermo Scientific TRACE 1300 Gas Chromatographic with SSL inlet, Flame ionization detector, auto sampler, Chromeleon software and HP-5 MS column with 30 m x 250µm ID x 0.25µm phenyl methyl silox 325 ^{0}C. Injection port and detector temperature is 260 and 300 ^{0}C, respectively.1µl was injected in split mode. The carrier gas (N2,99.999 purity) flow-rate was kept constant during the run at 1ml min^{-1}. Nitrogen (30 ml min^{-1}), hydrogen (40 ml min^{-1}) and dry air (400 ml min^{-1}) were used as auxiliary gases for the flame ionization detector.

Sample preparation and procedure

A 1.0 mg/mL stock solution of methamphetamine was prepared using methamphetamine CRM with chloroform containing phenylbenzylamine (PBA) at the concentration of 0.8mg/mL as the internal standard. Two standard series were prepared. Firstly, methamphetamine standard series of 10mg/L, 20mg/L, 30mg/L, 40mg/L and 50mg/L. Secondly, a series of concentrations ranging from50mg/L to and 400mg/L inclusive, concentrations were prepared using the aforementioned stock solution. These solutions then injected to GC- FID along with the stock solution at a concentration of 1.0 mg/mL. The solution was kept at 4±1°C when not in use and warmed to room temperature before use. Calibration curves of area methamphetamine/area PBA versus concentration methamphetamine/concentration PBA were constructed. A known weight of methamphetamine powder from each sample was dissolved in a 25 mL solvent mixture consisting of chloroform with PBA as the internal standard. These samples were injected to GC-FID, and purity of methamphetamine was calculated using the constructed calibration curve.

RESULTS AND DISCUSSION

Gradually developed blue color for the Simon's test indicated the presence of methamphetamine in powders in all samples. In the Marquis test, the development of an orange to brown color indicated the presence of methamphetamine in the powder from all the samples. The R_f value obtained for the methamphetamine CRM and that of the powder was the same, thus indicating the presence of methamphetamine in all samples. FTIR analysis was conducted for further confirmation of methamphetamine in the test samples.

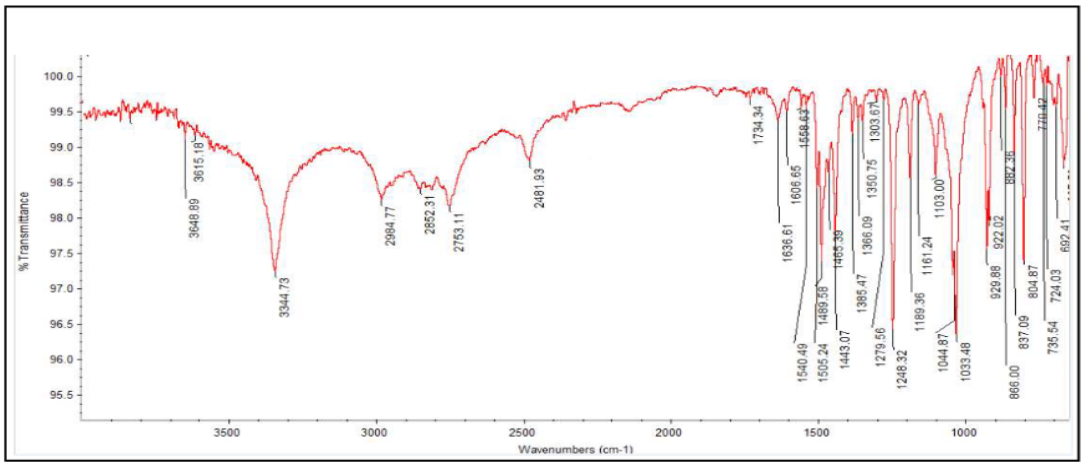

Figure 1. IR spectrum of the methamphetamine CRM

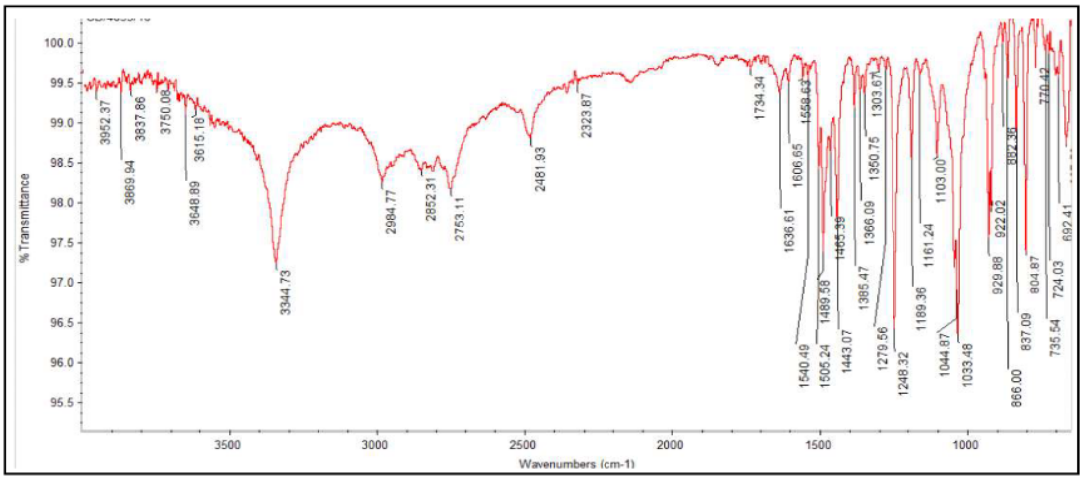

Figure 2. IR spectrum of the methamphetamine sample

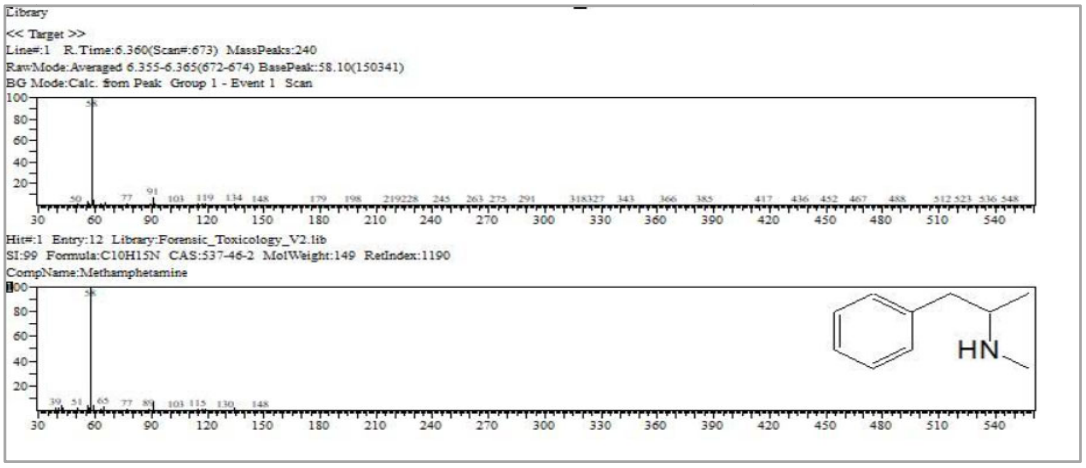

Figure 3. Mass Spectrums Obtained for the test Sample with the Library Match

Initially, methamphetamine CRM was analyzed using FTIR. Subsequently, the samples were analyzed. Figure 1 and Figure 2 illustrate the IR spectrum of standard methamphetamine and one of the test samples, respectively. The values of 3342 cm^{-1} and 3345 cm^{-1} were observed due to N-H stretching vibration, while the values of 2985 cm^{-1} and 2986 cm^{-1} were attributed to CH_3, CH_2, and CH stretching vibrations. Additionally, the values of 1490 cm^{-1} and 1465 cm^{-1} were assigned to aromatic C=C stretching vibrations, and the value of 1248 cm^{-1} was attributed to C-N stretching vibration in methamphetamine. These vibration frequencies are characteristic of both the test samples and the methamphetamine CRM.

The GC-MS results illustrate in the Figure 3confirmed the presence of methamphetamine with respect to methamphetamine CRM, in all test sample. The mass peak m/z at 58,91 and 149 which are characteristic for methamphetamine molecule were observed in the mass spectrums for the sample.

Method development and optimization

To optimize the chromatographic analysis for enhanced performance, several key parameters were fine-tuned. This involved selecting the most suitable column, determining the

concentration range based on methamphetamine levels in illicit drug powders, optimizing oven temperature programming, selecting an appropriate internal standard, diluent and establishing linearity [7]. The method development for methamphetamine was based on its chemical properties. Methamphetamine is a polar molecule and, therefore, a polar solvent chloroform was used as the diluent. Phenylbenzylamine was selected as internal standard. The methodology for developing GC-FID parameters based on the boiling point. Various temperature programs, flow rate and split ratio were investigated. The end of this investigation, the optimal temperature program, flow rate and split ratio were selected for a good resolution. The best operating parameter of GC-FID for methamphetamine analysis was given in Table 1.

Table 1. Operating Conditions for methamphetamine analysis by GC-FID

Column	30 m x250 µm ID x 0.25 m HP-5 MS
Injection	Split: 100:1
Injector Temperature	260 °C
Carrier Gas	Nitrogen at 1 mL/min flow rate
Oven Temperature Ramp Program	Initial Temperature :110 °C Start Time :4 minutes Temperature Rate :10 °C/min Final Temperature :280 °C Final Time :1 minute
Detector Temperature	300°C
Analysis Time	24.1 minutes

The solvent, column and acquisition parameters were chosen to be starting point for the method development. Typical chromatogram obtained with standard methamphetamine is presented in Figure 4.

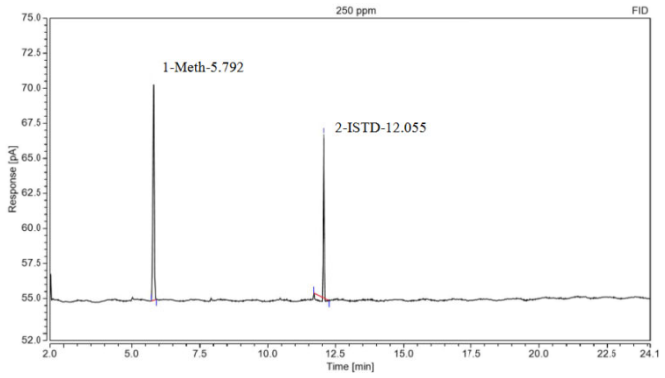

Figure 4. GC-FID chromatogram of methamphetamine

Method validation

Validation of the method was performed according to International Conference on Harmonization (ICH) guidelines [8] by means of linearity, precision, limit of detection and limit of quantification.

Linearity

Linearity describes the ability of the analytical method to obtain the test results directly proportional to the concentration of analyte in the test sample within a given range. The linearity of the analytical method is confirmed by the coefficient of regression of the calibration curve. Calibration curves were constructed by plotting peak area ratio versus concentration of methamphetamine in the range of 10-50 mg/L and 50-400 mg/L. The correlation coefficient valve (r^2) was used to evaluate linearity of develop method. Calibration curves were shown in Figure 5 and Figure 6.

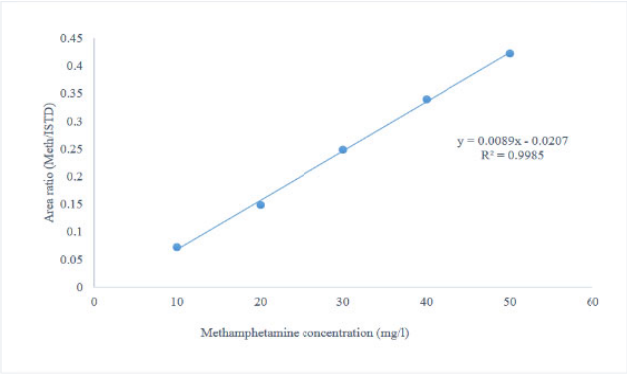

Figure 5. Calibration curve of methamphetamine (10 mg/L to 50 mg/L)

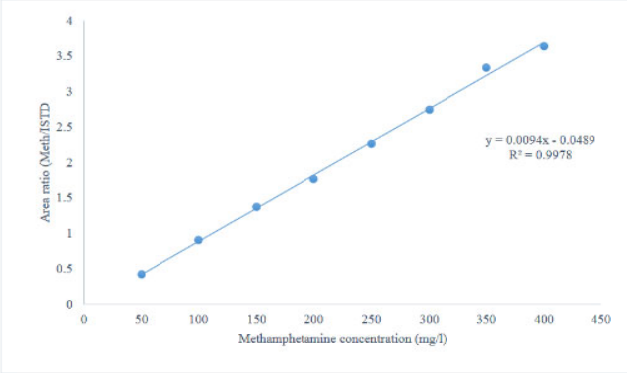

Figure 6. Calibration curve of methamphetamine (50 mg/L to 400 mg/L)

The data were subjected to statistical analysis using linear regression model, the regression equation and correlation coefficient are given in Table 2.

Table 2. Regression equation and correlation coefficient

Working range	Regression equation	Correlation coefficient (r^2)
50- 400 mg/L	y = 0.0094x – 0.0489	0.9978
10- 50 mg/L	y = 0.0089x – 0.0207	0.9985

Precision

Precision is the closeness of measurements when repeated analysis is carried out on a homogenous sample. Precision is a determined at three different levels of calibration. The precision of an analytical method is expressed as the Standard Deviation (SD) and Relative standard Deviation (RSD).RSD is used to evaluate precision of a set of replicate data(n=10). RSD were calculated for three concentrations of methamphetamine. Table 3shows calculated RSD of methamphetamine.

Table 3. Relative standard deviation of methamphetamine

Concentration range	Relative	Standard	Deviation (RSD)
	100 mg/L	250 mg/L	400 mg/L
50-400 mg/L	1.30	0.75	1.73

Limit of Detection (LOD)

LOD is the lowest amount of analyte in a test sample that can be detected under experimental conditions. LOD can be calculated using LOD = mean + 3 SD (Standard Deviation).

Ten replicates of 5 mg/L concentration methamphetamine standard were carried out to determine the LOD and LOQ. Table 4shows the resultant concentration of methamphetamine, mean, standard deviation, LOD and LOQ. LOD was 5.32 mg/L.

Table 4. Mean, standard deviation, LOD and LOQ results for concentration of methamphetamine

Injection No	Concentration (mg/L)
1	5.0857
2	5.0788
3	4.9507
4	4.9056
5	4.8732
6	5.1045
7	5.1432
8	5.0322
9	5.1507
10	5.0954
Mean	5.0420
Standard deviation	0.0936
LOD	5.32
LOQ	5.98

Limit of Quantification (LOQ)

LOQ is a lowest amount of an analyte in a sample that can be quantified with acceptable precision and accuracy. LOQ can be calculated using LOQ = mean +10 SD. LOQ value was 5.98 mg/L.

Quantification of methamphetamine

The percentages of methamphetamine in forty samples were determined within the calibration range of 50 mg/L to 400 mg/L using a validated GC-FID method. The purity of methamphetamine in the forty samples ranged from 14% to 96%.

Conclusion

This study involved examining forty samples to identify methamphetamine and determine its purity. Through screening tests and TLC analysis, all samples were initially identified as containing methamphetamine, which was subsequently confirmed via FTIR and GC-MS analysis. The GC-FID method was developed using optimal chromatographic parameters, including an HP-5 MS column (30 m x 250 µm x 0.25 µm) and a temperature program starting at 110°C for 4 minutes, then ramping up at 10°C/min to 280°C, with nitrogen as the carrier gas at a flow rate of 1.0 ml/min. Validation of this method for quantification analysis of methamphetamine demonstrated good performance in terms of linearity, relative standard deviation, limit of detection (LOD), and limit of quantification (LOQ). The method exhibited an LOD of 5.32 mg/L and an LOQ of 5.98 mg/L, indicating satisfactory sensitivity. Consequently, this method was successfully applied to quantify methamphetamine in the samples, revealing purities ranging from 14% to 96%. The GC-FID method proved to be relatively rapid, simple, precise, and suitable for routine forensic analysis.

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