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| Complete List of Authors: | Subramanian, Selvakumar; ISRO, Chemical testing Lab / SPROB<br>Somanathan, N; Polymer Laboratory, Central Leather Research Institute<br>Kami Reddy, Audisesha Reddy; ISRO, SPROB |
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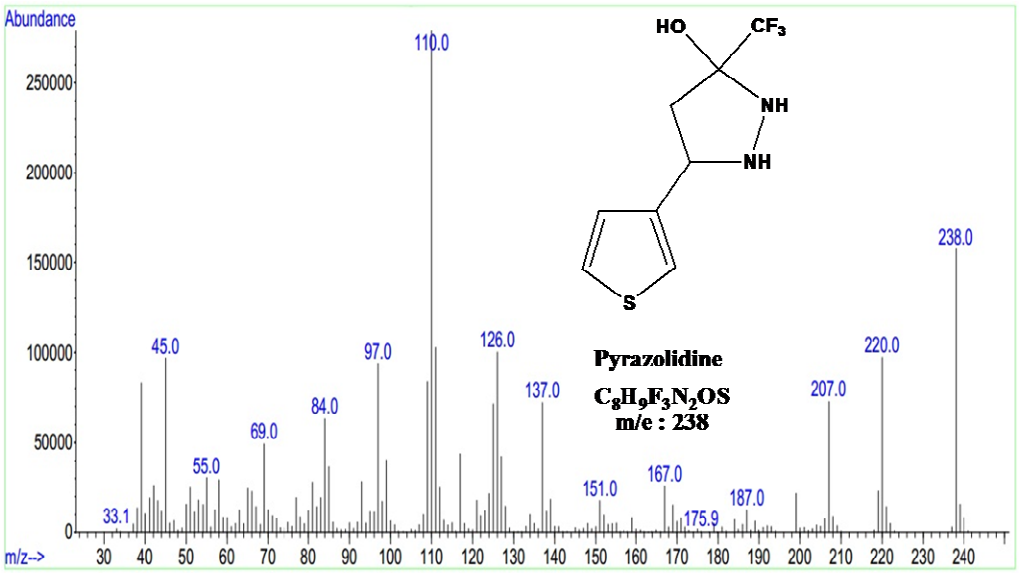
# Graphical Abstract



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PAPER

# Ambient Single Step Derivatization with $CF_3$ enone of thiophene to Determine Propellant Grade Hydrazines: A Study by GC and GC-MS

**Selvakumar Subramanian,[*ab] Somanathan Narayanasastri[*b] and Audisesha Reddy Kami Reddy [a]**

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A simple, highly selective and rapid gas chromatography method (Packed column with Flame Ionization detection) has been developed for the determination of hydrazines (Hydrazine, Monomethylhydrazine and hydrazine in the mixture of 1,1-dimethylhydrazine and hydrazine hydrate (ratio-75:25) in organic medium. This method is based on the derivatization of hydrazine (at ambient) with 1,1,1-trifluoro-4-(3-thienyl) ($CF_3$ enone) in the absence of catalyst / buffer which leads to the formation of corresponding pyrazolidine/pyrazoline/pyrazole. The organic derivatives thus formed are then detected and confirmed for their presence by GC–MS. The GC method provides good resolution between $CF_3$ enone and its derivatives with total analysis time of 20 min. Concentration of $CF_3$ enone and derivatization time are optimized to determine hydrazines in the concentration range of 0.4 mM to 0.2 M. The calibration curves based on peak areas of $CF_3$ enone and its derivatives showed good linearity with $r^2 \approx 0.999$ for the working range and the precision was found to be less than 1% for hydrazine, MMH and hydrazine in UH25. Recovery was found by standard addition method. Under the established condition, limits of detection were 20 µM for hydrazine, 10 µM for MMH and 20 µM for hydrazine in UH25. Tolerance limit for interfering amines was also found. Advantage of this method is selective detection and determination of hydrazine in UH25 mixture as 1,1-Dimethylhydrazine present in UH25 cannot be derivatized with $CF_3$ enone.

## 1 Introduction

Hydrazine and its derivative mono methyl hydrazine (MMH) are important both individually and in mixtures (UH-25-a fuel blend by weight of Unsymmetrical Dimethyl hydrazine and hydrazine hydrate in the ratio of 75:25) as rocket fuels due to their strong reducing nature. As they are highly reactive, they are widely used in chemical syntheses of explosives, military fuel cells and as chain extenders in the polymerization of urethanes. Though hydrazines are extremely useful chemicals for industrial and propellant applications, they are extremely hazardous. In space industries, large volumes of these hydrazines are shipped or transported every year as there is enormous increase in frequency of launches worldwide. The routine handling of these fuels occasionally results in the accidental spillage. The major problems observed with these hydrazines are that they can be absorbed through skin, affect blood production, cause liver and kidney damages. Due to their basic nature, they can cause dermatitis and severe burns if spilled on the skin. Exposure to high concentrations causes convulsions and possibly leads to death. They are known for their carcinogenic nature and can have heavy impact on human beings who are being exposed to these toxic compounds. Adverse health effects on people living near hazardous waste sites caused by hydrazines have been described by many researchers[1-6]. Hydrazine has a cancer risk level of $10^{-6}$ with an air concentration of 0.2 ng / lit and with a drinking water concentration of 10 ng / lit. Monomethyl hydrazine is a known mutagen and a suspected human carcinogen. National Institute of Occupational Safety and Health (NIOSH) has a permitted exposure limit (PEL) of 0.08 mg/m$^3$ ceiling and OSHA has a PEL of 0.35 mg/m$^3$ ceiling (skin) for MMH. Both hydrazines were listed by NIOSH as compounds immediately dangerous to life or health (IDLH).

Because of these considerable toxicological effects and industrial significance, the determination of these hydrazines at micro-levels is of great interest and practical importance. As they are explosive, toxic and carcinogenic in nature, many methods for detection (followed by determination) of these hydrazines have been proposed by many researchers to protect the personnel working in such a hazardous chemical environment. These include spectrophotometry[7-10], fluorescence[11-18], gas

[a] *Chemical Testing Lab, Solid Propellant Space Booster Plant, SDSC-SHAR Centre, Indian Space Research Organization (ISRO), Sriharikota 524124, Andhra Pradesh, India. Fax: +91-8623-225154; Tel: +91-8623-223013; E-mail: selvakumar.s@shar.gov.in; kumarreka@hotmail.com*

[b] *Polymer Division, Central Leather Research Institute (CLRI), Council of Scientific and Industrial Research (CSIR), Adyar, Chennai 600020, Tamil Nadu, India. Tel: +91-44-24437189; E-mail: nsomanathan@rediffmail.com*

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chromatography[19-28] and liquid chromatography[29-31] methods.

Out of these methods, GC has been widely used for hydrazines analysis because of its inherent advantages of simplicity, high resolving power, high sensitivity, short analysis time and low cost. Though GC of hydrazines is specific one, it is very difficult to obtain symmetrical hydrazine peaks even with Teflon supports in the columns.[19] It is due to their strong polar nature which results in sample adsorption. The detection limit for detection of these toxic compounds by GC has been improved by several orders using flame ionisation detection (FID). However, the FID is blind to hydrazine as it combusts hydrazine to form nitrogen and water. Alkylhydrazines such as monomethyl hydrazine and unsymmetrical hydrazine give only a weak signal because of the presence of low carbon content in them[2].

Derivatization is a popular technique for overcoming such type of problems. The main purpose of derivatization is to reduce the polarity of the amino group present in hydrazines and to improve GC properties of derivatives such as volatility, selectivity, sensitivity and separation[32]. Hence to improve GC properties for detection of these hydrazines by FID, they are converted to organic derivatives with high carbon (or even some halogen) content. Several of derivatization based GC methods for the determination of hydrazines have been reported in the past. They are mainly based on derivative formation from 2,4-Pentanedione[19], 1,1,1-trifluoroacetylacetone[20], pentafluoro benzaldehyde[21,22],p-nitrobenzaldehyde[23,24],Ethylchloro formate[25], acetone[26], o-phthalaldehyde[27]. (Table 1) These methods are selective either to hydrazine or MMH or UDMH only. Simultaneous application of derivatizing agents to both hydrazine and its methyl analogue (MMH) with their corresponding end products was not possible in these cases as the reactivity for both hydrazine and MMH is similar.

**<Insert Table 1>**

In addition to this, some of these methods are more time consuming[21,23,24,33] with poor recovery records obtained from non universal detections such as Electron Capture Detector[22] (ECD), Nitrogen Phosphorous Detector[21,23] (NPD). There is a need for catalyst for such type of reactions with pre-concentration step requirement[25] and high temperature application[20,27] or extraction of derivative with low boiling solvents is involved. Separation of interferences from main derivative is also tedious task in these cases. Some other potential problems with such derivatization procedures include the formation of unwanted derivatives, the presence of unchanged derivatization reagents as they affect during the analysis and a requirement for non aqueous reaction conditions.

Further to the above drawbacks, derivatizing agent such as acetone[34] and derivative of acetone are very much volatile in nature and there is every possibility of their loss during the concentration of volatile agent and its derivative leading to erratic determination of hydrazines. Moreover, they have minimal retention on GC column which is a major disadvantage for any GC analysis. Other derivatizing agents often need hydrophobic solvents in which hydrazines are immiscible.

Hence, α,β-unsaturated ketones with a trifluoromethyl group ($CF_3$ enone), an organofluorine compound was opted to meet the above challenges due to their special features such as solubility in high boiling solvent, high reactivity of its double bond and carbonyl group, the formation of stable fragments $CF_3C(OH)N$- with hydrazines and the stereo selectivity for the addition of nucleophiles (hydrazines). $CF_3$ enones have become valuable synthons for many synthetic purposes. Reactions of these enones are very much uncharacteristic of non-fluorinated unsaturated ketones.

Their extensive utilization for the synthesis of trifluoromethyl containing heterocycles was started only quite recently. But the use of these enones for the application to hydrazines is considered to be unexploited. In the case of the hydrazine and MMH, its reaction results in the formation of the corresponding pyrazolidines/pyrazolines/pyrazoles which are different.

The formation of pyrazolidines/pyrazolines/pyrazoles by the room temperature (RT) reactivity of active carbonyl group and double bond at 3-position of thiophene moiety {3-Butenone (E)-1,1,1-trifluoro-4-(3-thienyl)}with hydrazines in organic medium is the characteristic one for hydrazines.[35-42] This derivatization reaction is quick and quantitative at RT. Spectrophotometric method reported earlier by us[7] is based on the above principle and found to have a much higher sensitivity and supporting evidence for earlier findings. It has been applied successfully to the determination of hydrazine and MMH in acetonitrile medium. But this method works on the principle that identification and hence quantification of compounds is possible only at a particular wavelength that absorbs these compounds. As a result, reactivity difference between hydrazine and MMH with $CF_3$ enone could not be done. As GC analysis of these compounds separates complex mixtures of organics and allows individual compounds to be identified and quantified by a detector which is under use. As there is unequivocal identification requirement, a mass spectrometer (MS) coupled to the GC column was also employed in the present study. In this manuscript, we proposed a GC method which involves a very sensitive single step derivatization technique. This technique can be utilized to determine hydrazine and MMH individually (in trace levels) and for selective determination of hydrazine in UH25 mixture. In addition to that, we have described GC-MS technique for qualitative detection of $CF_3$ enone and its derivatives with hydrazine and MMH.

Here, we attempted to make use of such a simple and inexpensive derivatizing reagent for the GC determination of hydrazines which could be of very much analytical interest for quality control. GC method presented here, has desirable analytical properties (sensitivity, precision, selectivity and wide linear range) as well as being widely available for its application in any common labs which use universal detection technique like FID.

## 2 Experimental

### 2.1 Materials and Reagents

1,1,1-trifluoroacetone and Thiophene-3-carboxaldehyde (3-Thienaldehyde) were sourced from Sigma Aldrich, USA. Hydrazine (Purity: 99.7 % by GC), MMH (Purity: 99.6 % by GC), UH-25 (UDMH-74.2%; Hydrazine Hydrate-25.4 % by GC) are of propellant grade. Other chemicals including acetonitrile

used in the synthesis and characterization were sourced from Merck, India.

### 2.2 Characterization

FT-IR spectra of the samples were recorded on ABB MB 3000 Fourier transform infrared spectrometer by coating the sample on NaCl disc. NMR spectra were recorded by using JEOL ECA 500 MHz high resolution liquid state NMR spectrometer. Spectrophotometric measurements were performed on spectrophotometer (Model - Techcomp-8500) with 1 cm quartz cells. Agilent 6890 gas chromatograph with a split /split less injector (Agilent Technologies, USA) was used for GC-MS analysis. The analytical column was BP-1 MS column (100% dimethylpolysiloxane, 60 m, 0.25 mm I.D x 0.25 µm film thickness). Samples were injected in the split mode with a split ratio of 1: 100. The carrier gas (Helium) was passed through the column at constant pressure mode (44 psi). Temperature program was used to resolve the peaks. Initial temperature was at 120 °C, raised to 250 °C at the rate of 10 °C / min. All mass spectra were obtained using Agilent 5973 instrument. The ion source was operated in the electron ionization mode (EI: 70 eV, 230 °C). Full scan mass spectra (m/z 40-400) were recorded for the identification of the analytes at high concentration of standard solutions. The experiments for determination of hydrazines after derivatization were performed on Chrompack - CP-9001 GC equipped with FID. The GC column utilized for the above purpose was 10 % SE-30 (packed column-2 m X 1/8") on chromosorb-WHP, Mesh range - 60/80 (Make - Chromatopak, Mumbai). Maitre software (version 2.5) was used to control the instrument. It was also used to collect and process the experimental data. All the data (inclusive of calibration data) were obtained from the plots using the program Microcal Origin 7.0.

### 2.3 Preparation of Solutions

Solutions of $CF_3$ enone with concentrations of 2.5, 5, 10, 12.5, 20, 25 mM were prepared in acetonitrile medium. These solutions are stable for minimum ten days if they are kept at 5 °C under closed conditions. Derivative solutions after formation with hydrazine and MMH are stable for only one day.

Stock solutions of hydrazine, MMH and UH25 in acetonitrile medium (1 M) were prepared. Solutions with concentrations of 0.1, 0.4, 2, 4, 10, 20, 40 mM, 0.1, 0.2 M were prepared from each of the stock solutions. The standard ASTM method for Hydrazine (D1385-07) was used to standardize its concentration in its pure form and in UH25 mixture. Here, spectrophotometric measurements were carried out at 458 nm. The same method was utilized to standardize the concentration of MMH. Here, spectrophotometric measurements were carried out at 462 nm[7].

### 2.4 Synthesis and characterization of 3-butenone (E)-1,1,1 tri fluoro-4-(3-thienyl)

Synthetic procedure adopted earlier by our group has been followed with slight modification for the synthesis of $CF_3$ enone. Glacial acetic acid and piperidine were used in catalytic amount (1:1) with a solution of 3-thienaldehyde (0.05M) in dry benzene under nitrogen atmosphere. Trifluoroacetone (44.8g - 0.40 M) in eight parts (instead of 5 parts tried in our earlier synthesis) [7] to improve the yield (self condensation of Trifluoroacetone reduces the yield) and the mixture was stirred at RT till completion of the reaction. Reaction mixture was quenched with saturated ammonium chloride solution and washed with sodium bisulphite solution to remove unreacted aldehyde followed by water wash to neutral pH. The resultant solution was dried over sodium sulphate and concentrated under vacuum. Pure compound is eluted with n-Hexane.

Yield 65 % ; $^{13}$CNMR (in CDCl3) 180.5, 143.2, 137.1, 132.8, 127.9,125.2, 116.4; $^{1}$H NMR (CDCl3) - 6.8 (d, J=15.4, 1H); 7.4 (m, 2H-aromatic); 7.8 (m, 1H-aromatic); 7.9 (d, J =16, 1H).

GC-MS data shows that $CF_3$ enone is eluting at 8.6 min (Individual injection of $CF_3$ enone solution-Fig.S4 of ESI†) with m/e-206 (Mass spectra in Fig. 1) for which fragmentation pattern for $CF_3$ enone is shown in FP-01 of ESI.

**< Insert Figure 1 >**

FT-IR spectra of $CF_3$ enone in acetonitrile taken (in NaCl disc) shows five peaks, 1309 (aromatic–CH stretching), 2923 and 2854 (aliphatic–CH stretching), 1712 (>C=O stretching), and 1600 $cm^{-1}$ (olefinic –C=C stretching). The peaks identified are consistent with previously published data[7]. UV–Vis spectroscopy analysis of $CF_3$ enone in acetonitrile shows three peak maximas (201, 229 and 320 nm).The peaks identified are consistent with previously published data[7]. FTIR study of derivatives of hydrazine, MMH showed that there is a complete disappearance of carbonyl vibration at 1712 $cm^{-1}$ and C=C bond vibration at 1600 $cm^{-1}$ followed by the appearance of C=N bond vibration at 1668 $cm^{-1}$ . The changes occurred due to hydrazine and MMH were already explained in our earlier study[7]. As the changes noticed for UH25 derivative was similar to that of hydrazine, spectra for the same is not shown here.

## 3 Results and discussion

### 3.1 Chemical Structures and Derivatization Mechanism by GC-MS analysis

Chemical structures of $CF_3$ enone, hydrazine, MMH and their possible derivatives are given in the schemes 1 and 2. Strong electron accepting nature of trifluoroacetyl group present in $CF_3$ enone causes its reaction with hydrazines to proceed faster at RT. Here, the possible reaction is heterocyclization, which is proceeded by two routes. The first route is a primary Michael addition of a nucleophile to enone with subsequent cyclization and the second route is a primary addition of a nucleophile at the carbonyl group followed by cyclization. This might be due to similar nucleophilicity of the two nucleophilic centers of hydrazine.[35]

Scheme 1 depicts probable derivatives of $CF_3$ enone after reaction with hydrazine. As per this scheme, heterocyclization reaction results in the formation of pyrazolidine (eluting at 11.87 min. - Fig. 2, m/e-238 by GC-MS - Fig. S5 of ESI†for which fragmentation pattern is given in FP 03 of ESI) and pyrazoline (eluting at 14.06 min. - Fig. 2, m/e-220 by GC-MS in Fig. 3 for which fragmentation is given in FP 02 of ESI). The formation of

pyrazoline is due to dehydration of pyrazolidine.[7] Change in the peak area of pyrazolidine depends on the change in concentration of hydrazine. When concentration is increased, there is corresponding increase in the peak area of pyrazolidine also. Here, pyrazoline is formed in small quantity and there is no significant change in its peak area even for the addition of higher concentration of hydrazine. The results provide strong supporting evidence for our theoretical explanation[7] and for the findings of other researchers.[35-37]

**<Insert Scheme 1 & Figures 2, 3>**

Scheme II indicates the reaction of MMH with $CF_3$ enone forming four derivatives. Reaction with MMH leads to the formation of pyrazolines I and II[35] (eluting at 10.41 min and 12.74 min - Fig. 4, m/e - 234 by GC MS for both derivatives – shown in Figs. S6 (of ESI†) and 5 respectively for which fragmentation patterns are shown in FP 04 and 05of ESI). These derivatives are regio isomers of pyrazoline (formed in ~1:3 ratio)[35] which are resultant of heterocyclization followed by dehydration. This result also provides supporting evidence for our earlier study.[7]

**< Insert Scheme 2, Figures 4 and 5 >**

In addition to pyrazoline isomers, two regioisomers of pyrazoles (eluting at 13.91 min and15.80 min - Fig. 4, m/e-232 by GC-MS for both derivatives – shown in Fig. S7 and S8 respectively for which fragmentation patterns are shown in FP 06 and 07 of ESI) are formed[38-40] as a result of heterocyclization which is unexpected and not suggested by us in our earlier study.

Regiochemistry observed in both cases of pyrazoline and pyrazole is due to two different nucleophilic centers of methyl hydrazine[35]. There is a possibility of attack of one nucleophile (either $-NH_2$ or $-NCH_3$) at both the double bond and the carbonyl group followed by dehydration which results in the formation of pyrazoline/pyrazole isomers. Direct formation of pyrazoline by spontaneous removal of water might be due to significant increase in nucleophilicity of MMH by replacement of one hydrogen atom in hydrazine by methyl group. The vertical comparison of hydrazine and MMH studied by T. A. Nigst et al.[43] shows that substituted nitrogen in MMH is activated by a factor of 11(MMH/Hydrazine).[8]

Formation of Pyrazoles[40-42] (Derivatives III and IV) is not dependent on the concentration of MMH indicating that there will be no significant change in the peak areas of these derivatives. As there is corresponding changes in the formation of derivative I and II for the change in the addition of MMH, these derivatives are used for calibration purpose. It is to be noted that there was very small but proportional change in the formation of derivative I. In both GC chromatograms (obtained from reaction mixtures of hydrazine and MMH with $CF_3$ enone), unreacted $CF_3$ enone is eluting at 8.6 min.

UDMH of UH25 does not react with $CF_3$ enone to form pyrazolidine/pyrazoline/pyrazole. Heterocyclization is not favored with UDMH which may be due to totally unbalanced nucleophilic centres of UDMH namely $-NH_2$ and $-N(CH_3)_2$.

Unsymmetrical replacement of two hydrogen atoms in hydrazine by methyl groups further increases its nucleophilicity[43,8] at one side of $NH_2$-$NH_2$ which is not a favorable one for heterocyclization. Due to this reason, hydrazine present in UH25 mixture alone forms derivatives. This is confirmed by matching with the retention times of hydrazine derivative peaks. As the conversion pattern for UH25 was similar to that of hydrazine, GC-MS analysis was not carried out for UH25 derivative.

### 3.2 Optimization of variables

Scouting experiments were performed for the selection of column with several test conditions (Details are given in ESI). SE-30 column is found to be suitable for present study as it is superior in quality and has silicone which is inert and resistant for the chemical attack by derivatives of hydrazines with good thermal stability.[44] The polar –Si-O-Si- bond in the stationary phase material improves resolution of $CF_3$ enone from its derivatives. Temperature programming was found to be better option for good resolution and quantification. Experimental conditions thus optimized and standardized are given as follows: Carrier gas and flow rate: Helium, 40ml / min ; Injection port temperature : 210 °C ; Detector temperature : 225 °C ; Oven temperature : 120-200 °C at 8 °C ; Injection volume : 2 µL ; Run time : 15 min.

Effect of temperature over reactivity of $CF_3$ enone with hydrazines and its completion time was carried out at 50 °C at different time intervals. Application of temperature results in fast completion of reaction with similar pattern of peaks obtained for the trials at RT. No major change was observed in the peak areas of derivatives or $CF_3$ enone. Hence, the entire study is planned at RT. Swift decrease in the area of $CF_3$ enone peak and hence increase in the area of $CF_3$ enone derivative peaks were observed for the addition of higher concentration of hydrazines (as there is correspondingly better reactivity). Lower concentration of hydrazines (as there is correspondingly less reactivity) caused comparatively small but similar change in the areas for $CF_3$ eone and its derivatives. Out of the various concentrations of $CF_3$ enone tried (2.5, 5, 10, 12.5, 20 and 25 mM), concentration of 20 mM was optimized for further studies. After fixing the concentration of $CF_3$ enone, concentration in the range of 0.2 M to 0.4 mM for hydrazine or MMH or UH25 (depending on the study requirement) was tried for derivatization at RT. Ratio fixed for $CF_3$ enone and hydrazine (or MMH or UH25) in acetonitrile was 1:1. Though the reaction was completed within 5 min, derivatization time was tried with 10, 15 and 20 min. As there was no noticeable change in the areas of $CF_3$ enone and its derivatives after 5 min., derivatization time was fixed as 5 min. Derivatization procedure as stated in section 3.3 was followed for further studies.

### 3.3 Derivatization Procedure

Into a series of 10 ml volumetric flasks were added 1 mL of $CF_3$ enone solution of fixed concentration and 1 mL of hydrazine solution (or MMH or UH25) in the concentration range of 0.2 M to 0.4 mM. The solution was mixed thoroughly and was kept aside for 5 min to ensure the completion of the reaction at RT. This solution (Injection volume-2 µL) is injected into SE-30 column. Duplicate is also performed for determination of

hydrazines.

### 3.4 Analysis of $CF_3$ enone and its hydrazine derivatives by GC-MS

Capillary column used for detection of $CF_3$ enone and its hydrazine derivatives by GC-MS was BP-1 MS column. Temperature program was similar to that of packed column which is explained in section 3.2. Chromatogram of $CF_3$ enone and its hydrazine derivatives formed after hydrazine addition is shown in Fig.3. As similar stationary phase was used for both packed column and capillary column, peak pattern was found to be similar for the analysis by these columns. When sample solution (obtained after room temperature derivatization with hydrazine) is injected into the column, there is an elution of three peaks. One is due to $CF_3$ enone with retention Time (R.T.) - 8.6 min and other two are indicating the derivatives formed after hydrazine addition (R.T. for derivative I : 11.87 min) (R.T. for derivative II -14.06 min). Mass spectras for $CF_3$ enone and its hydrazine derivatives I and II are shown in Figs. 1, S5 (of ESI) and 3 respectively.

### 3.5 Determination of hydrazine by GC based on $CF_3$ enone and its hydrazine derivative

#### 3.5.1 Linearity, Detection limit, Repeatability and Reproducibility

$CF_3$ enone was found to decrease on increasing the concentration from 0.4 mM to 0.2 M and this gives a linear relationship between its area and hydrazine concentration as shown in Fig.S1A of ESI. Peak areas of the derivative (pyrazolidine) were found to increase on increasing hydrazine. This gives a linear relationship between the peak areas of the derivatives and the concentration of hydrazines as shown in Fig.S1B of ESI.

Based on this observation, calibration methods have been established for the determination of trace level hydrazine. Calibration graphs of peak areas of $CF_3$ enone versus concentrations were plotted (in two ranges- shown in Fig.6 A and B). As the change in the area of $CF_3$ enone is in the decreasing order for increase in the concentration of hydrazine, negative trend is observed for the calibration plot based on $CF_3$ enone. Calibration graphs of peak areas of $CF_3$ enone derivative (pyrazolidine) versus concentrations were plotted (in two ranges- shown in Fig. S9 A and B of ESI).Each point on the curve is the mean of two injections.

**<Insert Figure 6>**

The regression equations with correlation coefficients are given in the respective curves which indicate best linearity. Limit of detection (LOD) measured with a signal-to-noise ratio of 3:1) was found to be 20 µM. Relative standard deviation (R.S.D) for five replicate determinations of pyrazolidine derivative obtained from 0.02 M hydrazine using $CF_3$ enone is 0.7%. Same derivative sample was analyzed by three different analysts to see the analyst bias. Intra-day variations ($n$ = 3) in terms of the peak areas and R.T.s were measured for the same and the RSD was found to be 0.5 – 1.5 % for both.

#### 3.5.2 Analysis of $CF_3$ enone and its MMH derivatives by GC-MS

Procedure adopted for the determination of hydrazine was followed by replacing hydrazine with MMH and the observations were recorded. Chromatogram of $CF_3$ enone and its MMH derivatives after addition MMH is shown in Fig.6. Due to similar stationary phase, peak pattern was found to be similar for the analysis done by packed and capillary columns. When sample solution (obtained after room temperature derivatization with MMH) is injected into the column, there is an elution of five peaks inclusive of $CF_3$ enone peak. Peak eluting at 8.6 min is due to $CF_3$ enone which is confirmed individually as well as with hydrazine trials. Addition of MMH in the concentration range of 0.4 mM-0.2 M leads to the formation of four derivatives. Retention times for these four peaks (10.44, 12.74, 13.93 and 15.80 min) do not match with those of either $CF_3$ enone (8.6 min) or its hydrazine derivatives (11.87 and 14.06 min). This indicates that the derivatives formed from MMH are totally different from that of hydrazine. Mass spectras for MMH derivatives are shown in Figures 7, 8, 9 and 10 respectively.

### 3.6 Determination of MMH by GC based on $CF_3$ enone and its MMH derivatives

#### 3.6.1 Linearity, Detection limit, Repeatability and Reproducibility

Similar to hydrazine trials, area of $CF_3$ enone was found to decrease on increasing MMH concentration from 0.4 mM to 0.2 M resulting in a linear relationship as shown in Fig.S2A of ESI. Peak areas of pyrazolines I and II were found to increase on increasing MMH concentration which results in a linear relationship as shown in Fig.S2 B and C of ESI. Based on this observation, calibration methods have been established for the determination of trace level MMH. Calibration graphs of peak areas of $CF_3$ enone versus concentrations were plotted covering the above said range (in two ranges- shown in Fig.13A and B). Similar to calibration plots of hydrazine, negative trend was observed for the calibration plots based on $CF_3$ enone. This is due to linear decrease in its area for increase in MMH concentration. Calibration graphs of peak areas of MMH derivative-I (pyrazoline-I) of $CF_3$ enone versus concentrations were plotted (in two ranges- shown in Fig.14 A and B). Calibration graphs of peak areas of MMH derivative-II (pyrazoline-II) of $CF_3$ enone versus concentrations were also plotted covering the same range (in two ranges- shown in Fig.15A and B).Each point on the curve is the mean of two injections. Regression equations with correlation coefficients are given in the respective figures. Best linearity was observed similar to hydrazine trials. Limit of detection (LOD) obtained is 10 µM. R.S.D for five replicate determinations of derivative I obtained from 0.02 M MMH using $CF_3$ enone is 0.9%. Same sample was analyzed by three different analysts. Intra-day variations ($n$ = 3) in terms of the R.T. and the peak area were measured for the same and RSD was found to be 0.5 – 1.8% for both R.T. and peak area.

**<Insert Figures 13-15>**

### 3.7 GC MS analysis of $CF_3$ enone and its UH25 derivative

Procedure adopted for the determination of hydrazine was followed by replacing hydrazine with UH25 and the observations were recorded. Addition of UH25 in the concentration range of 0.4 mM - 0.2 M leads to the formation of derivatives similar to hydrazine. When derivative sample solution is injected into the column, there is an elution of four peaks. One is due to $CF_3$ enone (Retention time - 8.62 min) and another two peaks are indicating the derivatives formed after UH25 addition. Retention times of these peaks match with those of hydrazine derivatives. This indicates that the derivatives formed from UH25 are mainly due to hydrazine.

### 3.8 Determination of hydrazine (in UH25) by GC based on $CF_3$ enone and its UH25 derivative

#### 3.8.1 Linearity, Detection limit, Repeatability and Reproducibility

Similar to trials with hydrazine, peak area of $CF_3$ enone decreases on increasing the UH25 concentration (0.4 mM - 0.2 M) resulting in a linear relationship and hence negative trend as shown in Fig.S3A of ESI. Peak areas of the derivative were found to increase on increasing UH25 concentration showing linear relationship as shown in Fig.S3B of ESI. As done in the case of hydrazine and MMH trials, calibration graphs were plotted in the concentration range of 0.4 mM - 0.04 M based on $CF_3$ enone and its hydrazine derivative (from UH25) as shown in Fig.16A and B. It is to be noted that linear relationship was not found in the concentration range of 0.04 M -0.2 M.(Fig. S3 A and B)

**<Insert Figure 16 >**

Regression equations with correlation coefficients are given in the respective figures. Best linearity was observed similar to hydrazine and MMH trials. Limit of detection (LOD) obtained from practical trials is 20 µM. R.S.D for five replicate determinations of pyrazolidine obtained from 0.02 M UH25 using $CF_3$ enone is 0.5 %. Derivative sample is analysed by three different analysts to see the analyst bias. Intra-day variations ($n$ = 3) in terms of the R.T. and the peak area were measured for the same and the RSD was found to be 0.5 – 1.2 %.

### 3.9 Application of the method

To evaluate the analytical applicability of the method, the proposed procedure was applied for determination of hydrazines by standard addition method in acetonitrile medium. As a result, known amount (in two different trials) of hydrazine, MMH and UH25 was spiked into solution with known concentration. Results (shown in table 2) confirm the reliability of the proposed method.

**<Insert Table 2>**

To study the selectivity of the procedure, the effect of different amines such as methyl amine, butyl amine, dibutyl amine, triethyl amine and aniline on the determination of 0.02 M hydrazine and MMH was tested under established conditions. This effect was investigated by adding a known amount of the test specie in concentration ranging up to 0.4 mM to the hydrazine and MMH derivative solutions obtained from 0.02 M of respective hydrazine. Tolerance is considered as the interferent concentration that produces an error smaller than 5% in the analyte determination. The tolerance limits for all the above amines is less than 0.01 mM which shows that the method has a good tolerance level for tested amine.

## Conclusions

An accurate and reliable GC method has been developed to determine concentration of hydrazines in an organic medium. The method is simple, single step, selective, sensitive, reproducible and rapid in comparison to other derivatization methods. Excellent linear relationship between concentration of $CF_3$ enone and its derivatives (with hydrazine, MMH and hydrazine in UH25) with respect to FID response was demonstrated with minimal detectable concentration of 10 µM. Unlike other GC based derivatization methods, determination of hydrazines by this method can be carried out not only with respect to derivatives but also derivatizing agent. Moreover, it provides strong supporting evidence for the earlier findings. This method has the following other advantages also. (a) There is no catalyst requirement (b) advantage of room temperature application (c) Less concentration of $CF_3$ enone (20 mM) is sufficient (d) Selective for hydrazine in UH25 (e) If $CF_3$ enone is used as a liquid chemosorbent in chemo sorption tubes to determine the concentration of hydrazine in air, there will be no need of time consuming and cumbersome steps such as desorption and derivatization (f) Incorporation of $CF_3$ enone functional group into other heterocyclic compounds such as carbazole where there is a possibility of attaching at more than one position or oligomers and conjugated polymers with such functional groups will lead to enhancement of reactivity towards hydrazines. This present manuscript forms the basis for such an improved selective detection of hydrazines.

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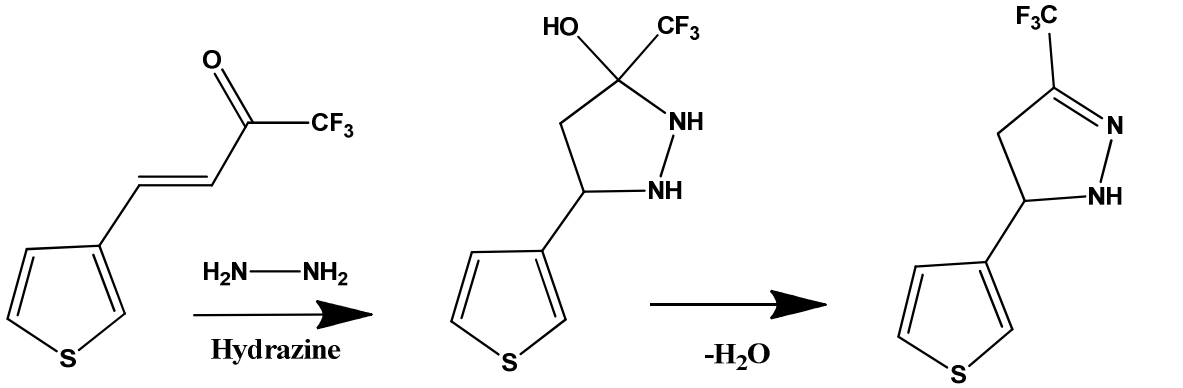
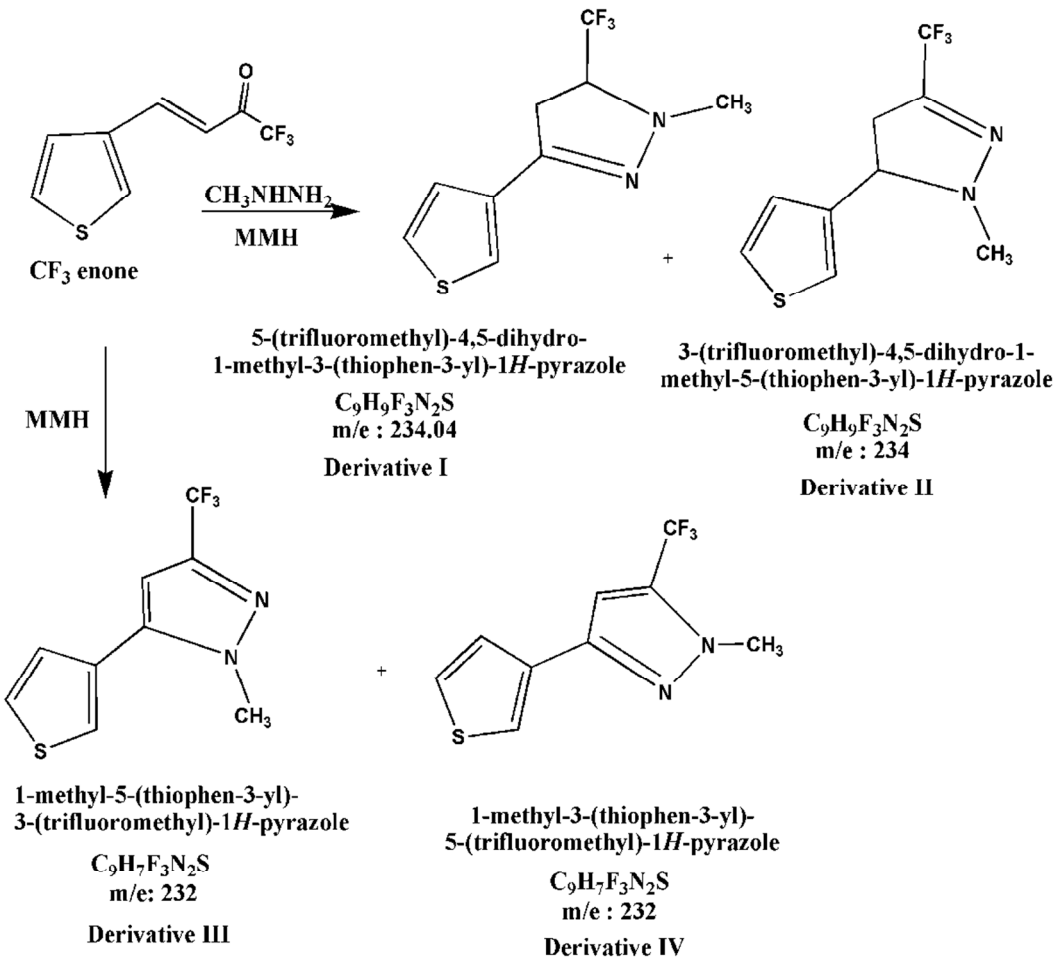



**$CF_3$ enone**
**$C_8H_5F_3OS$**
**m/e : 206**
**(*E*)-1,1,1-trifluoro-4-(thiophen-3-yl) but-3-en-2-one**
**Derivatizing agent**
**R.T. 8.6 min**

**Pyrazolidine**
**$C_8H_9F_3N_2OS$**
**m/e : 238**
**3-(trifluoromethyl)-5-(thiophen-3-yl)pyrazolidin-3-ol**
**Major Product**
**R.T.11.87 min**

**Pyrazoline**
**$C_8H_7F_3N_2S$**
**m/e : 220**
**3-(trifluoromethyl)-4,5-dihydro-5-(thiophen-3-yl)-1*H*-pyrazole**
**MajorProduct**
**R.T.14.06 min**

**Scheme 1 Derivatization of $CF_3$ enone with hydrazine**




**Derivative I (R.T.-10.41 min) & II (R.T.-12.74 min.) – Pyrazolines I & II- Formation ratio - 1: 3**

**Derivative III (R.T.-13.91 min.) & IV (R.T.-15.80 min.) – Pyrazoles I, II- Minor Products**

**Scheme 2 Derivatization of $CF_3$ enone with MMH**

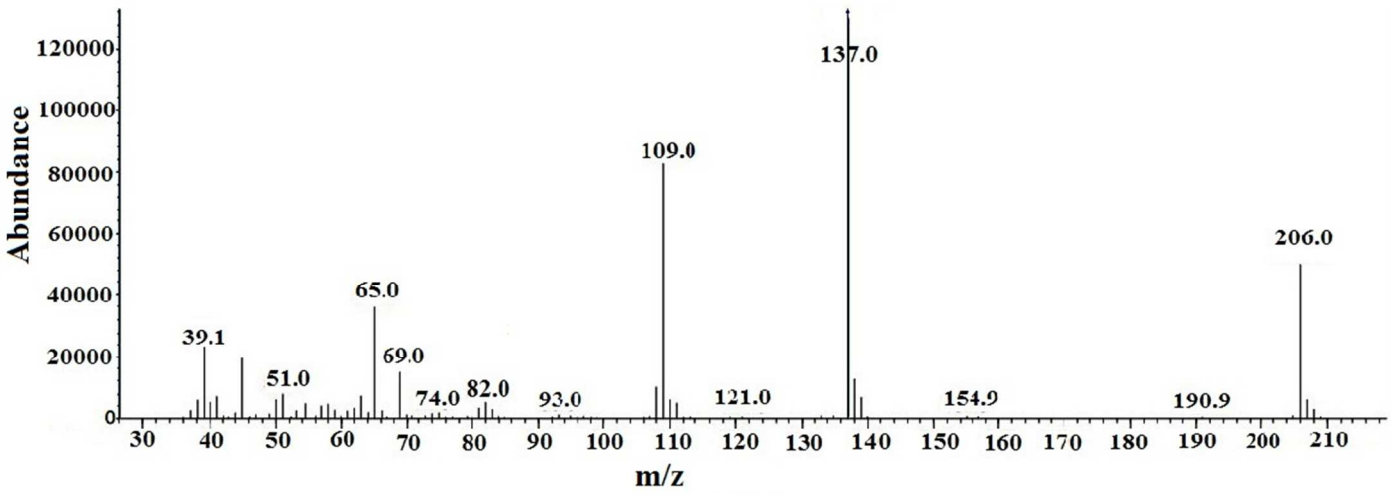



**Fig. 1.** Mass spectra for the peak eluted at 8.6 min. ($CF_3$ enone)

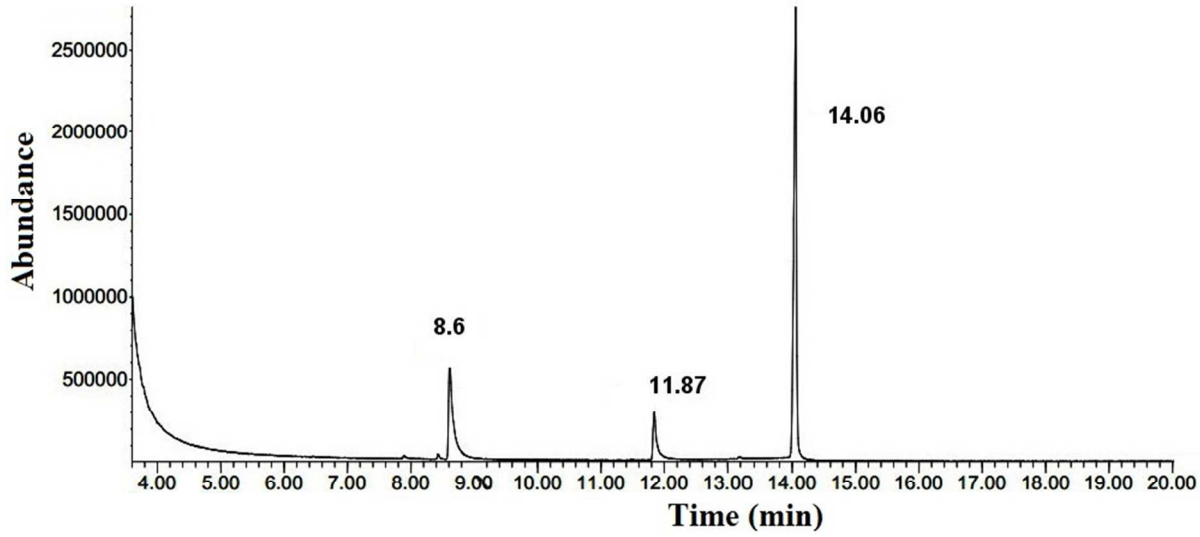



**Fig. 2** Chromatogram for $CF_3$ Enone and its hydrazine derivatives

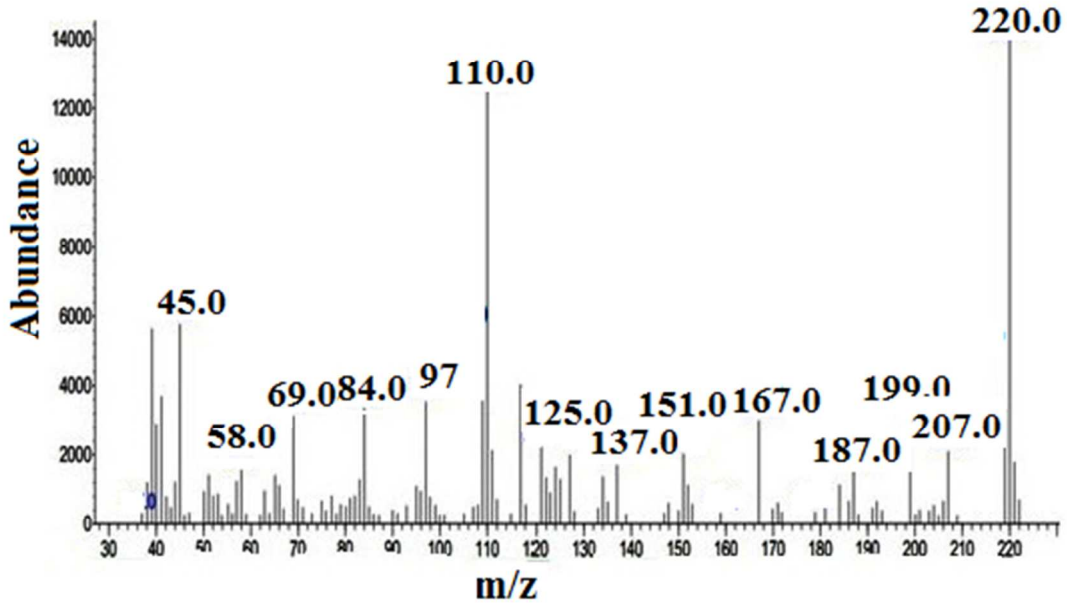



**Fig.3.** Mass Spectra for the peak eluted at 14.06 min. (Pyrazoline)

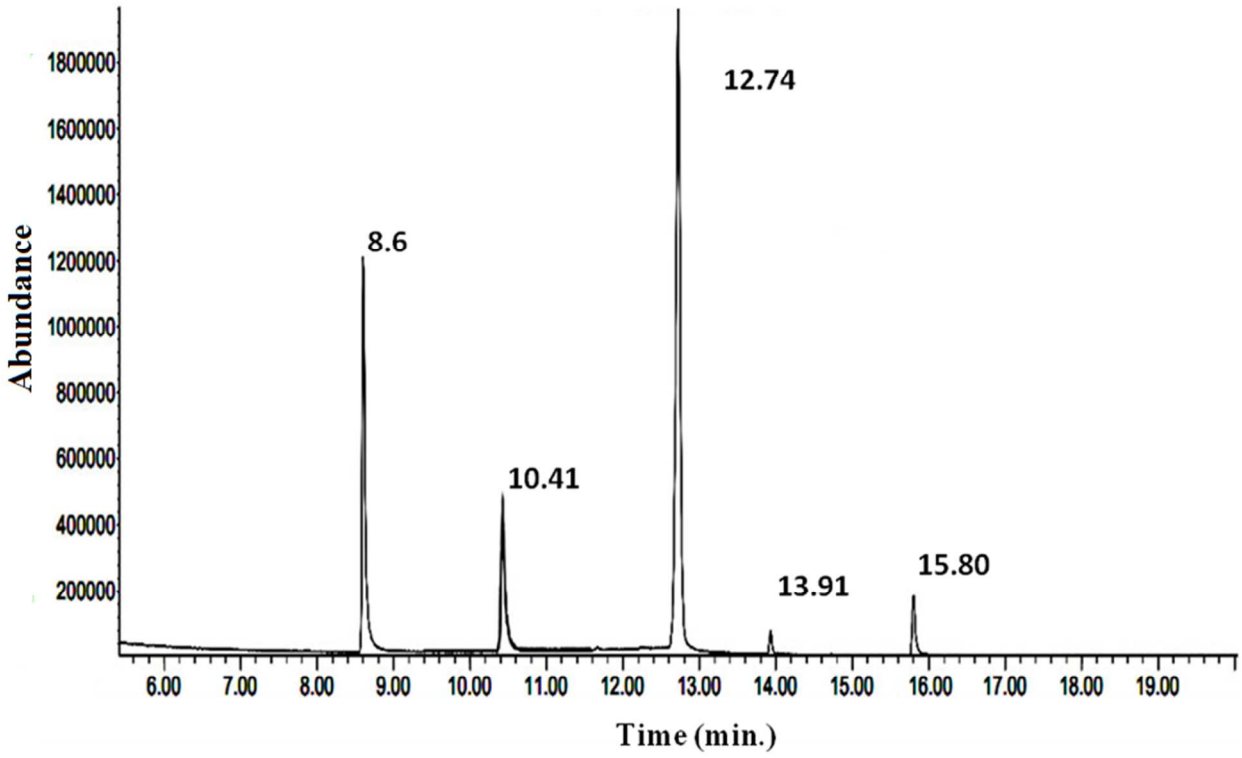



**Fig. 4** Chromatogram for $CF_3$ Enone and its MMH derivatives

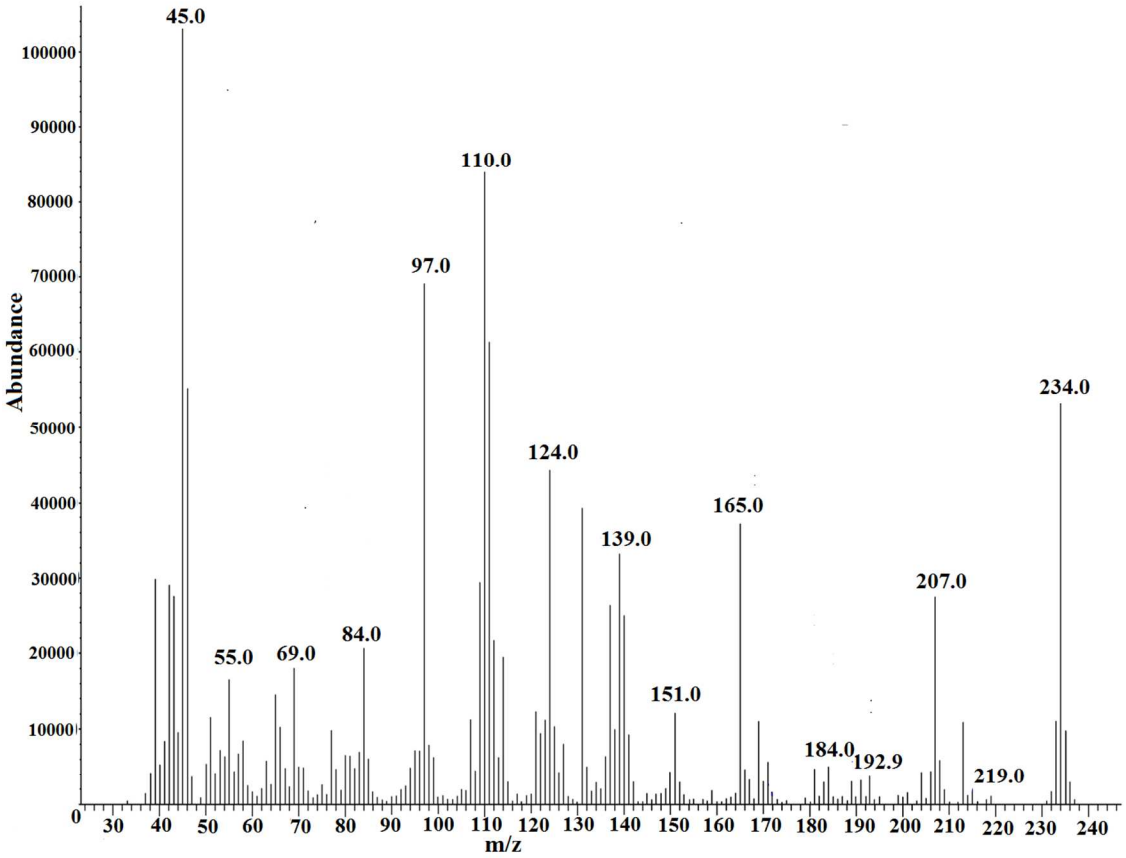



**Fig.5** Mass Spectra for the peak eluted at 12.74 min. (Pyrazoline II – MMH derivative-II)

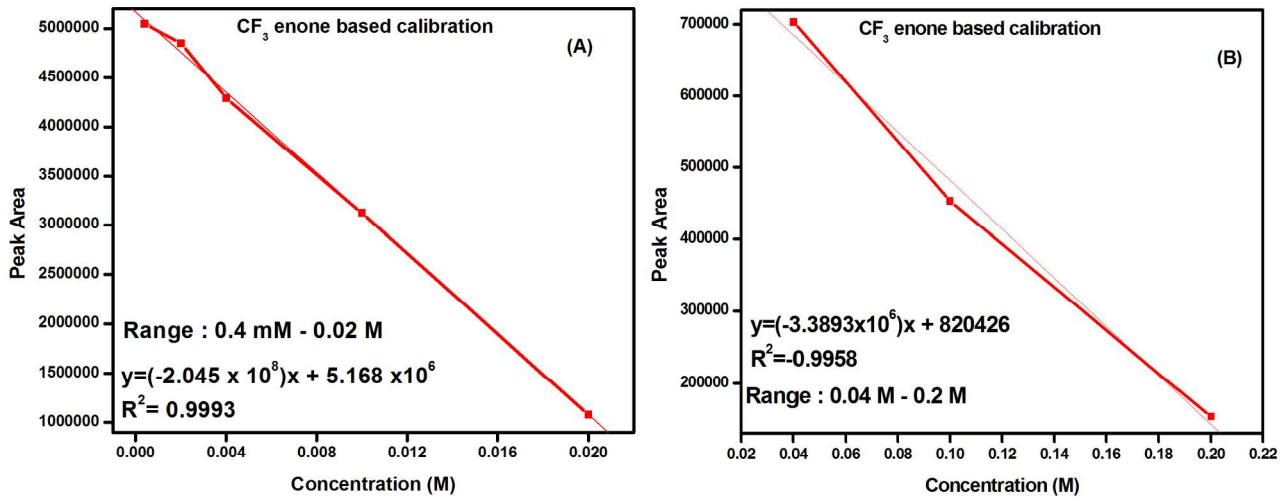



**Fig.6.** Calibration graphs for the determination of hydrazine based on $CF_3$ enone in the concentration range of 0.4 mM-0.02M (A) and 0.04 M – 0.2 M (B)

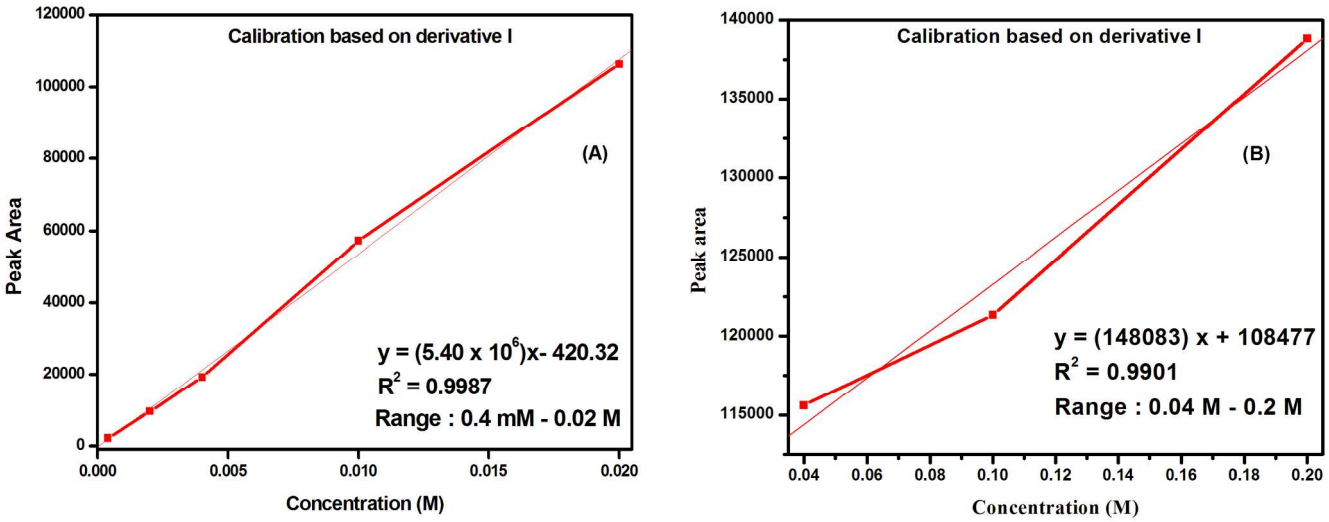



**Fig.7.** Calibration graphs for the determination of MMH based on Pyrazoline - I in the concentration range of 0.4 mM-0.02M (A) and 0.04 M – 0.2 M (B).

## LIST OF TABLES

**Table 1.** GC Determination of hydrazine and its methyl derivatives by derivatization

| Derivatizing agent<br>Extracting solvent<br>& Test conditions | Determined substance in medium | Detection | LOD | Ref. |
|---|---|---|---|---|
| 2,4-Pentanedione<br>Water, 5°C | Hydrazine in water<br>MMH in water | FID | 0.1 mg $L^{-1}$<br>0.1 mg $L^{-1}$ | 19 |
| 1,1,1- trifluoroacetylacetone<br>Chloroform; 15 min; 75°C; | Hydrazine in pharmaceuticals | FID | 0.001 mg $L^{-1}$ | 20 |
| Pentafluoro benzaldehyde<br>Ethyl acetate; 30 min ; 25°C | Hydrazine in urine, plasma, liver | NPD | 5 ng $Kg^{-1}$ | 21 |
| Pentafluoro benzaldehyde<br>n-Hexane; 20 min; 25°C | Hydrazine in maleic acid hydrazide | ECD | 50 µg / $Kg^{-1}$ | 22 |
| 4-nitrobenzaldehyde<br>n-Hexane; 30 min ; 80 °C | UDMH in water | NPD | 0.03 µg $L^{-1}$ | 23 |
| 4-Nitrobenzaldehyde<br>n-Hexane ;30 min. ; 37 °C | UDMH in soil | FID | 10 µg / $Kg^{-1}$ | 24 |
| Ethyl chloroformate<br>Chloroform; 15 min at 25°C | Hydrazine in pharmaceuticals | FID | 0.002 µg $L^{-1}$ | 25 |
| Acetone<br>Dichloromethane; 10 min ; 100 °C | Hydrazine in vapour phase analysis | MS | 0.0001 µg $L^{-1}$ | 26 |
| O-Phthalaldehyde<br>Methylene chloride;20 min;<br>70 °C; Buffer pH - (Initial - 2;<br>Final - 9.5) | Hydrazine in drinking water and surface water | MSD | 0.002 µg $L^{-1}$ | 27 |
| $CF_3$ enone<br>No extraction ; 10 min.; RT | Hydrazine<br>MMH<br>Hydrazine (in UH25)<br>(all in organic medium) | FID | 0.05 mg $L^{-1}$<br>0.10 mg $L^{-1}$<br>0.05 mg $L^{-1}$ | This work |

**Table 2 Precision & Accuracy of the method based on $CF_3$ enone peak (n=3)**

| Hydrazine | | | MMH | | | Hydrazine in UH25 | | |
|---|---|---|---|---|---|---|---|---|
| Taken * (mg/ml) | Added* (mg/ml) | Found + RSD % | Taken * (mg/ml) | Added* (mg/ml) | Found + RSD % | Taken * (mg/ml) | Added* (mg/ml) | Found + RSD % |
| 3.3 | 1.1 | 4.4 ± 0.05 | 2.45 | 0.91 | 3.36 ± 0.05 | 2.2 | 1.1 | 3.3 ± 0.03 |
| 2.2 | 1.2 | 3.4 ± 0.03 | 4.67 | 0.45 | 5.12 ± 0.04 | 1.2 | 3.3 | 4.5 ± 0.05 |

* mg/ml of respective hydrazine added to CF3 enone ;

n = No. of determinations for particular concentrations tried.