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A Headspace Gas Chromatographic Method for Determination of Formic acid Content in Isosulfan Blue and Various Drug Substances

NILESH TAKALE[1,2], NEELAKANDAN KALIYAPERUMAL[2], GOPALAKRISHNAN MANNATHUSAMY[1] and RAJARAJAN GOVINDASAMY[1]*

[1]Department of Chemistry, Annamalai University, Annamalainagar, Chidambaram, Tamilnadu, India.
[2]Emcure Pharmaceuticals Ltd, Analytical Research Centre, Hinjawadi, Pune-411057, Maharashtra, India.
*Corresponding author E-mail: Mail: rajarajang70@gmail.com

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ABSTRACT

The Pharmaceutical industry uses formic acid in the manufacturing of various drugs or API. At the time of manufacturing of API formic acid is use as an oxidizing agent. Formic acid is the simplest carboxylic acid. It also called methanoic acid. Formic acid present in API at high concentrations is very hazardous but in low concentrations is very beneficial. The developed and validated method was short, precise, cost effective and reproducible with FID detector and easy to use. The method is a selective and superficial analytical method for determination of formic acid in different drug substances. We report here the development and validation study of headspace gas chromatographic method to determine formic acid in different drug substances we are reported here. As per this method, the drug sample was dissolved in 0.1% (v/v) of concentrated sulfuric acid in isopropyl alcohol (IPA) in a GC headspace vial and 0.1% (v/v) of concentrated sulfuric acid in isopropyl alcohol used as a diluent. A AB-Inowax capillary column (30 m x 0.32 mm I.D. and 0.5 μm film thickness) was used under gradient conditions with FID. The formic acid peak was well separated from all other solvents that are used in synthesis of particular drug substance. The LOD and LOQ of the method for formic acid are 82 ppm and 249 ppm respectively. Formic acid are low toxic class-III solvent as per ICH guideline.

Keywords: Formic acid, Drug substance, GC-HS, AB-Inowax GC column.

INTRODUCTION

Isosulfan blue Fig. 1 is well known indicator its work like methylene blue. It is a diagnostic agent to detect the cancer cell. Isosulfan blue is called as [4-[α-[p-(Diethylamino)phenyl]-2,5-disulfobenzylidene]-2,5-cyclohexadien-1-ylidene] diethylammonium hydroxide, inner salt, sodium salt[1]. It is an indicator used for lymphographic procedure. Isosulfan blue is a greenish blue powder and sparingly soluble in water. Molecular formula and molecular weight of Isosulfan blue is $C_{27}H_{31}N_2NaO_6S_2$ and 566.66 g/mol. It preserves in tight containers and store at 20°C to 25°C.

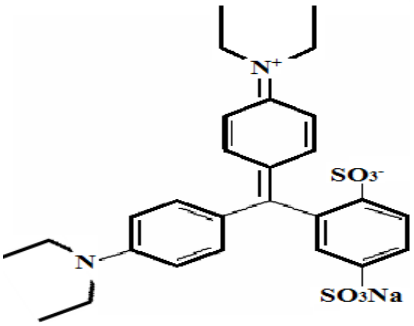

Fig. 1. Chemical structure of Isosulfan blue

The method development of formic acid is a very challenging task because formic acid is low UV active compound also it doesn't thermally ionize, therefore it is difficult to detect on gas chromatography. While it derivatized with sulfuric acid and isopropyl alcohol, it shows response on Gas Chromatography. Hence derivatized method is selected for analysis of formic acid in various drug substances.

Drug substance are synthesized with use of various raw materials, intermediates, catalysts, reagents and solvents. Formic acid was used as catalyst, reagent and solvents at various stages of synthesis of drug substance and its raw materials, intermediates. It can be mainly formed by oxidative degradation[2].

Formic acid is one of the low toxic class-III solvents which are not specified or quantified in pharmaceuticals till it exceeds the limit of 5000 ppm[3]. This limit is defined according to toxicological considerations only. However, lower levels of formic acid may cause instability of drug products as it can react with amino and/or hydroxyl groups in many pharmaceuticals. This leads to formation of the corresponding amides, as happened withvarencline, and/or esters degradation products. It can also modify formulation pH and form salts with basic drugs[4,5]. Moreover, formic acid has accelerated the degradation of the experimental drug FK480 by optical isomerization from the desired S-enantiomer to undesired R-enantiomer[6].

So many analytical methods have been applied for analysis of formic and acetic acids, including titration methods[7], HPLC[8], IC[9] and GC[10]. The outdated titration methods are normally not selective, and they show content of total acids[11,12]. Mostly varied results occurred. In the IC methods, so many precautions taking place at the time of analysis like clean the suppressor and stable the current with extensive and burdensome pre-treatment[13,14].

Formic acid is volatile and highly reactive. Isosulfan blue is less detector sensitive as well as low UV active. So, it is difficult to detect and determine formic acid readily. Many analytical methods have been developed to analysis formic acid such as colorimetric[15], spectrophotometric[16], GC, HPLC and ion chromatographic methods. Lots of these methods employ chemical derivatization before formic acid determination[17-20]. Colorimetric and spectrophotometric methods have not sufficient specificity and/or sensitivity. Ion exchange chromatographic methods have in adequate specificity because of the susceptibility to interfering ions that may be originated from the used reagents, solvents, glassware or from the sample itself[21]. Some HPLC and GC methods have not sufficient specificity and/or sensitivity. While others require multi steps and time-consuming sample preparation procedures. Many reported methods have been derivatized formic acid by alcohols before gas chromatographic determination[22-24].

In this method analysis of formic acid in different drug substances is very simple and fast. Sample preparation was very easy and quick, and under mild conditions chemical derivatization can be carried out. This method was very specific and sensitive.

The analysis of formic acid in different drug substances is very challenging work due to superior method is required for analysis. In this research, formic acid content is determining in different drug substances and its development and validation study is carried out. The main aim of this research is to develop superior and dedicated GC-HS method and it has to be used either "as is" or with slight modification for determination of formic acid in different drug substances. An initially we have taken drug substance and develop the GC-HS method for determination of Formic acid. Sample from five representative drug substances namely drug substance 1 (Isosulfan blue), drug substance 2 (Decitabine), drug substance 3 (Docetaxel), drug substance 4 (Trimethobenzamide Hydrochloride) and drug substance 5 (Febuxostat), their analysis conduct on initially developed GC-HS method. The synthesis of above mentioned five

drug substances has not the same raw materials, intermediates, catalysts, reagents and solvents. The main aim of this research is to find out the general or universal method to analyze the samples of five drug substances. GC-HS method has the essential selectivity and superiority to be used as a universal method to analyze formic acid in drug substances. Using a Isosulfan Blue drug substance to develop universal GC-HS method for determination of formic acid was effectively validated for its anticipated use.

The main goal of this study was to develop and validate a GC-HS method to quantify formic acid in drug substance, and to use it for in quality control laboratory.

METHODS AND MATERIALS

The drug substances taking from Emcure Pharmaceutical Limited, Hinjawadi Pune-411 057. 1-Methyl-2- pyrrolidone (NMP) was purchased from Spectrochem Pvt. Ltd. (Mumbai). Isopropyl alcohol, concentrated sulfuric acid and formic acid was purchased from Merck (Mumbai).

The AB-Inowax column (Stationary phase 100% polyethylene glycol with 0.5 μm film thickness 30 m x 0.32 mm I.D.) (Make: Abel Bonded).

Perkin Elmer 680 or equivalent gas chromatogram (GC) equipped with a Perkin Elmer Turbomatrix 40 headspace sampler with FID detector, and total chrome navigator software are used for data acquisition.

Diluent preparation

Dilute concentrated sulfuric acid (0.1% v/v) in Isopropyl alcohol.

Standard and sample solution preparation

Formic acid standard 0.25mg/mL was prepared by diluting formic acid to diluent. The drug substance sample solution was prepared by 50 mg/mL make up with diluent. A 1.0 mL of above standard, sample solution and 1.0 mL of 1-Methyl-2-pyrrolidone (NMP) added in to each separate 22 mL HS vial and placed on HS system for analysis.

Development of Method

The main goal of this method is to determine the formic acid content at low quantification level with better resolution and high sensitivity in different drug substance. GC-HS analytical technique is very superior because of in this technique only volatile compounds analyzing in HS vial. Hence, a GC-HS method was discovered for the quantification of formic acid in different drug substances. The AB-Inowax GC capillary column (100% polyethylene glycol stationary phase) has been use to determination of acids in drug substances, and that the reason this column was selected for development of method and validation study. Usually, In GC-HS method temperature programming is used for to achieve the resolution between different solvents. Since in this method a simple gradient column oven temperature program was discovered to analyze formic acid content. The diluent, temperature gradient program, split ratio, carrier gas flow, HS oven temperature, attenuation, range, transfer line temperature, needle temperature and other HS and GC parameters were explored and improved using formic acid standard or 100% spiked sample solution of formic acid.

Preparation of diluent for sample and standard solution

In the method development sample diluent plays more important role to achieve recovery and response of analyte peak. At the time of development so many diluents were used containing isopropyl alcohol (IPA), methanol (MeOH) and ethanol (EtOH) alone and sulfuric acid with combination. Formic acid peak was not detected in GC-HS technique so derivatized it, Formic acid derivatized with methanol, ethanol and isopropyl alcohol in presence with sulfuric acid (0.1 % v/v). Methanol peak and formic acid peak is not well resolved as compare to isopropyl alcohol and ethanol. The isopropyl alcohol and ethanol eluted approx. same retention time, Fig. 3. To achieve better resolution between formic acid peak and diluent peak to select the isopropyl alcohol for derivatization. Formic acid derivatization: Formic acid was derivatized with isopropyl alcohol in presence of sulfuric acid. The derivatization reaction was carried out in the headspace vial which was heated at 65°C for 15 min in the headspace incubator. Thus the diluent 0.1% Sulfuric acid in isopropyl alcohol use for method development. The 1-Methyl-2- pyrrolidone (NMP) was used for solubility of drug substance. This combination was studied under different HS oven temperatures like 65°C, 85°C and 95°C (with 100°C constant temperature of transfer line). under all these GC-HS conditions, the constancy of formic acid 100% spiked (250 parts per million) drug substance was evaluated and the result demonstrated that the 0.1% sulfuric acid in Isopropyl alcohol (v/v) diluent at a HS temperature 65°C shows the best superiority and specificity.

Optimization of Chromatographic conditions and Headspace parameters

For the optimization of gas chromatographic conditions like gradient column oven temperature program to achieve the peak shape of formic acid followed by a temperature ramp to 200°C to eluted the diluent peak (NMP). Since B. P. of formic acid is 100.8°C, the column oven temperature of GC was assessed at 35°C, 40°C, 50°C and 60°C using a formic acid (250 parts per million) 100% spiked drug substance. The retention time of formic acid is 3.7, 3.1 min, 2.5 min and 1.8 min at the gas chromatography column oven temperature at 35°C, 40°C, 50°C and 60°C, individually. The sensitivity of the 250 parts per million formic acid standard response was satisfactory (s/n ratio higher) for all parameters and conditions studied. For all above study it was concluded that, the column oven temperature at 35°C was selected for other method parameters optimization study. Since this parameter or condition found the good resolution between formic acid peak and diluent peak (IPA).

The main goal of method development was to achieve satisfactory sensitivity for low quantification level formic acid analysis. Split ratio plays key role in method sensitivity of formic acid. At the time of development for some GC injections various split ratios were studied respectively 3:1, 5:1 and 10:1. The satisfactory s/n was detected or observed using the 5:1 split ratio.

The GC and HS parameters are shown in Table 1. Using 250 (parts per million) ppm formic acid spiked drug substance to optimize better accuracy and specificity. Formic acid eluted at approx 3.7 minute. which was well resolved from other solvents, diluent and any other peaks. Fig. 2 shows that the chromatogram of the diluent, sample solution, limit of detection (LOD), limit of quantitation (LOQ), formic acid standard solution (250 parts per million) and spiked sample solution (250 parts per million).

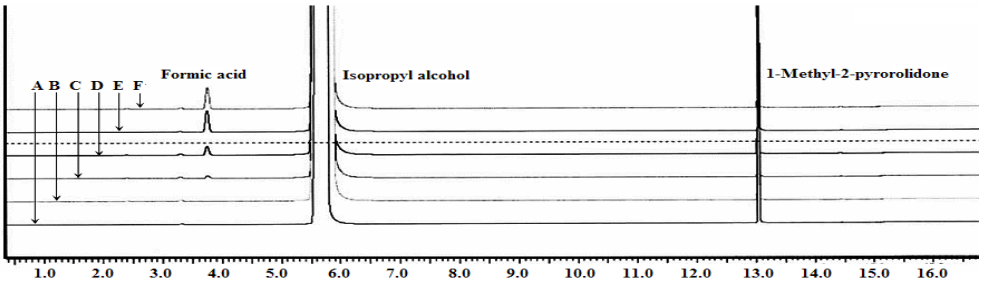

Fig. 2. The chromatograms of (A) diluent, (B) sample solution, (C) limit of detection (82 ppm), (D) limit of quantitation (249 ppm), (E) formic acid standard solution, (F) spiked sample solution

Table 1: GC instrument chromatographic conditions for Formic acid

Component	Specification
GC parameter	
Instrument	Perkin Elmer 680 or equivalent gas chromatogram (GC) equipped with a Perkin Elmer Turbomatrix 40 headspace sampler with FID detector
Column	AB-Inowax capillary column (30 m x 0.32 mm I.D. and 0.5 μm film thickness)
Column Oven temp.	35°C for 5 min increased to 200°C at 20°C/min hold for 5 minutes
Carrier gas	Nitrogen
Carrier gas Flow	1.5 mL/min
Injector temp.	200°C
Injector split ratio	5:1
FID temp.	250°C
Range	1
Attenuation	-3(8)
Headspace parameter	
HS oven temperature (°C)	65
HS needle temperature (°C)	80
Transfer line temperature (°C)	100
GC cycle time (in minutes)	38
Thermostat time (in minutes)	15
Pressurized time (in minutes)	1.0
Time of withdrawal (in minutes)	0.3
Time of injection (in minutes)	0.05
HS pressure (psi)	16.0
Shaker	On

Equation (1) shows the results found of the formic acid in different drug substance:

$$\text{Formic acid (ppm)} = \frac{(A_s - A_b)}{(A_{std} - A_b)} \times \frac{W_s}{100} \times \frac{5}{50} \times \frac{1}{W_t} \times \frac{P}{100} \times 10^6$$

Where, A_s and A_{std} = area of formic acid in sample and standard solution, individually. A_b = areas of formic acid in blank chromatogram. W_s = formic acid standard weight in mg/mL. W_t = weight of sample in mg. P = purity of formic acid. 10^6 = Parts per million Conversion factor.

RESULTS AND DISCUSSION

The optimized general formic acid content method was validated for drug substances. The method was validated for method precision, system precision, Intermediate precision, selectivity, linearity, accuracy, limit of quantitation (LOQ), limit of detection (LOD), range, robustness and solution stability.[25]

Selectivity

The method selectivity is one of the important parameters in validation study. In Isosulfan Blue drug substance so many solvents are used at the time of manufacturing. As shown in Fig. 2, there are no measurable peaks in the chromatogram of diluent and sample. In standard and spiked sample solution would interfere with formic acid in the chromatogram. In Fig. 3 shows that all solvent they are used in manufacturing of Isosulfan blue. The formic acid peak in the Fig. 3 is well resolved from all other solvent they are used in formation of drug substance. The RT of formic acid in the chromatogram of spiked sample solution and standard solution exactly match with each other. The formic acid peak was well resolved from all other solvents that are present in synthesis of Isosulfan blue. Fig. 3 shows the chromatograms of all solvents which are used in synthesis of Isosulfan blue. The formic acid peak is well resolved from all other solvents they are used in the synthesis of Isosulfan Blue. All residual solvents injected on GC-HS as per ICH limits. Hence it is proved that the developed method has satisfactory selectivity for used solvent. Since those solvents are well separated from formic acid, the good resolution between formic acid and all solvents in Isosulfan blue shows that the developed and validated method is a universal or general or unique for quantification of formic acid. This method is universal method for quantification of formic acid in different drug substances and that can be applied either “as is” or slightly changes.

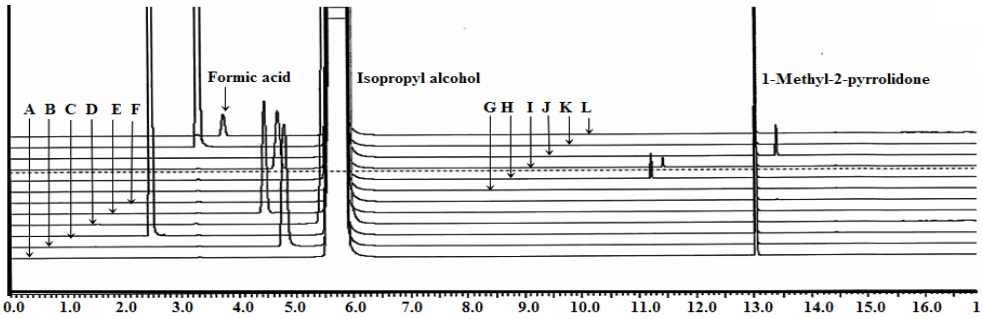

Fig. 3. The chromatograms of (A) diluent, (B) methanol (3000 ppm w.r.t sample conc.), (C) cyclohexane (3880 ppm w.r.t sample conc.), (D) ethanol (5000 ppm w.r.t sample conc.), (E) ethyl acetate (5000 ppm w.r.t sample conc.), (F) dichloromethane (600 ppm w.r.t sample conc.), (G) acetonitrile (410 ppm w.r.t sample conc.), (H) dimethylacetamide (1090 ppm w.r.t sample conc.), (I) acetic acid (5000 ppm w.r.t sample conc.), (J) nitrobenzene (1000 ppm w.r.t sample conc.), (K) acetone (5000 ppm w.r.t sample conc.), (L) formic acid standard (250 ppm w.r.t sample conc.)

Selectivity for the formic acid in five drug substances

The proposed method is universal method for formic acid on different drug substances that can be applied “as is” or slightly changes. The same method is applied for the analysis of five different drug substances that have different raw materials, reagents, solvents, intermediates and catalysts. The five drug substances are Isosulfan blue, Decitabine, Docetaxel, Trimethobenzamide Hydrochloride, Febuxostat respectively. Above mentioned five drug substances analyse on the same method and chromatograms of those as shown in Fig. 4. The chromatogram shows that the formic acid peak is well separated from other peaks and no any interference peak in the retention time of formic acid peak. Hence it is proved that method is applicable for the determination of formic acid

for those five drug substances. Since those five drug substances tested, the good sensitivity of the method for those five drug substances shows that this method is universal for formic acid in different APIs (drug substances) that can be applied either "as is" or with slightly changes.

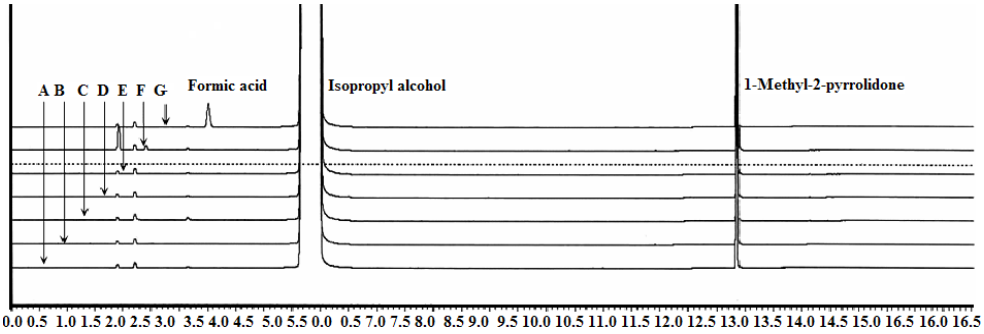

Fig. 4. The chromatograms of (A) diluent, (B) Sample solution of Isosulfan Blue, (C) Sample solution of Decitabine, (D) Sample solution of Docetaxel, (E) Sample solution of Trimethobenzamide Hydrochloride, (F) Sample solution of Febuxostat, (G) formic acid standard solution

Limit of detection(LOD) and Limit of quantitation (LOQ)

The linearity of formic acid was assessed from 50 parts per million to 3000 parts per million (eight different concentration levels with each level prepared in triplicate) in the nonappearance of drug substance. Plotted a graph of peak areas versus corresponding theoretical concentrations and the calculate the linear regression and find out the Limit of quantitation.

LOD=LOQ*0.33

Triplicate analysis (n=3) of 82 (parts per million) ppm formic acid standard and 249 (parts per million) ppm formic acid standard calculated the mean of s/n ratios of 15 and 125, individually. Based on the recovery data finalized the LOQ of above method parameters was 249 ppm. Therefore, the Limit of quantitation (LOQ) and the Limit of detection (LOD) was thus fixed at 249 parts per million and 82 parts per million, individually. These s/n value for LOD is not less than 3 (NLT~3) and LOQ is not less than 10 (NLT~10), which confirm the robustness of the method during its daily use in the Quality control.

Linearity, Recovery, Precision and Range

The linearity of formic acid was assessed from 250 parts per million (LOQ) to 7500 parts per million (150% level) (six different concentration levels with each level prepared in triplicate) without drug substance. Plotted a graph of peak area versus corresponding theoretical concentrations and the calculated the linear regression. This method correlation coefficient of formic acid standards without drug substance is 0.99952 (the acceptance criteria for correlation coefficient should be greater or equal to 0.99). The method is in line with slope of formic acid standards without drug substance are 206.9839.

As per analytical method in presence of drug substance (Isosulfan blue) analysing the triplicate preparation of formic acid standard at 250 parts per million to 7500 parts per million standard concentrations and calculate the %recovery. The %recovery was calculated from the found concentrations of formic acid divided by the added concentrations of formic acid. The acceptance criteria of recovery are 80% to 120%. In Table 2 shows the %recovery of six different levels including LOQ. The recovery results of six different levels are well with in acceptance criteria. Fig. 5 shows the chromatograms of those five drug substances 100% spiked sample solutions and they show approx. 100% recovery. 100% Standard solution (250 ppm w.r.t sample) spiked with those five drug substances and they show in Figure 5.

Table 2: Accuracy results of Isosulfan Blue from LOQ to 150% level

Level No.	Concentration in ppm	Recovery	SD	%RSD
Level-1 (LOQ)	250	95.68%	0.8423	0.88
Level-2 (50%)	2500	96.42%	2.7718	2.87
Level-3 (80%)	4000	95.30%	1.2573	1.32
Level-4 (100%)	5000	100.27%	2.1047	2.10
Level-5 (120%)	6000	97.93%	0.5198	0.53
Level-6 (150%)	7500	97.59%	0.5330	0.55

(n=3:triplicate injection of each level including LOQ to 150%)

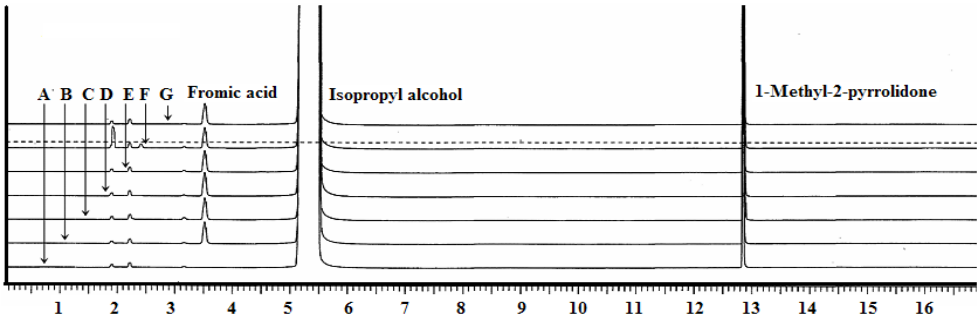

Fig. 5. The chromatograms of A (diluent), 100% Spiked (250 ppm w.r.t sample) sample solutions of B (Isosulfan Blue) C (Decitabine), D (Docetaxel), E (Trimethobenzamide Hydrochloride), F (Febuxostat) and (G) formic acid standard solution (250 ppm)

Precision

System precision was proven by injecting a six injection of formic acid standard (250 ppm). The %RSD of the six replicate standard injections is 1.76. Method precision was proven by injecting a six spiked samples using analytical method. The %RSD of the six replicate spiked sample injections is 1.44.

Intermediate precision was proven by injecting six spiked sample using different columns, analyst, days, instrument in same laboratory. The %RSD results of two different analysts, two different days, two different columns and two different instruments is 2.15.

The range 250-7500 parts per million (LOQ-150% level) meets the acceptance criteria of recovery, precision and linearity.

Stability of Solution

The solution stability of standard and sample were prepared fresh, 12 hrs. and 30 houre. The area of standard, cumulative % RSD of the standard, content in sample solution and cumulative % RSD of the sample is mentioned below Table 4a and 4b. The cumulative % RSD for area of standard preparation and results at each interval is less than 15.0.

Robustness

Robustness was demonstrated by performing, deliberately varied GC and headspace parameters. Table 5 shows robustness parameters like varied GC and HS parameters and their results. In robustness, Sample were tested by changing one method parameter at a time with injecting the 250 ppm formic acid standard spiked with drug substance sample solution.

Table 3: Comparison of Method precision and Intermediate precision results

ID	Formic acid content (ppm)	
	Method precision	Intermediate precision
Sample solution -1	4723	4628
Sample solution -2	4808	4919
Sample solution -3	4805	4585
Sample solution -4	4636	4848
Sample solution -5	4740	4680
Sample solution -6	4679	4822
Average (n=6)	4732	4747
SD (n=6)	68.2273	134.3905
%RSD (n=6)	1.44	2.83
Average (n=12)	4739	
SD (n=12)	101.9219	
%RSD (n=12)	2.15	

Table 4a: Area and cumulative % RSD of standard

Time (Hrs.)	Area of standard solution	Cumulative %RSD for Standard solution
Fresh	50567	–
12 Hrs.	51496	1.29
30 Hrs.	49853	1.63

Table 4b: Content and Cumulative % RSD of sample

Time (Hrs.)	Content in sample solution (ppm)	Cumulative % RSD for Standard solution
Fresh	4944	–
12 Hrs.	4799	2.10
30 Hrs.	4760	2.01

Therefore, Standard and sample of formic acid were stable for 30 h at room temperature

Table 5: Robustness study results of formic acid spiked in Isosulfan Blue with changing GC and HS parameters

Parameter	Condition	Retention time	Tailing factor	%RSD of standard preparation
Column lot	Lot 1	3.616	1.06	1.76
	Lot 2	4.313	1.10	1.01
Flow rate (mL/min)	1.3 mL/min	4.053	1.06	1.35
	1.7 mL/min	3.285	0.99	1.24
Initial oven	32°C	3.624	1.01	1.83
	38°C	3.627	1.03	1.34
HS oven temperature	63°C	3.856	1.00	1.02
	67°C	3.412	1.01	1.52
Shaker	On	3.616	1.06	1.76
	Off	4.539	1.10	1.36

CONCLUSION

In this research to develop universal or common method of GC-HS for Isosulfan blue and four different drug substances. This method is suitable for total five different drug substances for the quantification of formic acid. As per this method, the drug sample was dissolved in 0.1% (v/v) of concentrated sulfuric acid in isopropyl alcohol (IPA) in a GC headspace vial and 0.1% (v/v) of concentrated sulfuric acid in isopropyl alcohol used as a diluent. A AB-Inowax capillary column (30 m x 0.32 mm I.D. and 0.5 μm film thickness) was used under gradient conditions with FID. A total five different drug substance can be analyzing by use of this method on as is basis. Isosulfan blue full validation study performs in this paper. Remaining four drug substance analysis and 100% spiking sample recovery were tested in this article. Accuracy of six different levels including LOQ was found well with in acceptance criteria. All known solvents are well separated from the formic acid peak. The LOD for formic acid was 82 parts per million. This method was effectively validated with different parameters like recovery, linearity, robustness, precision solution stability etc. and it can be used for routine analysis in quality control department.

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Conflict of interest

None

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