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Research Article

Development and Validation of GC-FID Method for the Quantification of N-Iodosuccinimide

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ABSTRACT

A simple, rapid and cost effective method for N-Iodosuccinimide was developed and validated using gas chromatography with flame ionization detection (GC-FID). GC separation performed and NIS peak eluted about 4.19 min using a HP5 column 30mtX0.32 ID, film thickness 0.25 micron. Nitrogen was used as carrier gas at a flow rate of 1.8ml^{-1}min. After injection of the sample at inlet temperature 260°C, The temperature programs of the GC oven was as follows: 110°C hold for 4 min, increased to 240°C at the rate of 10°C^{-1} min and held for 3 min. Detector temperature was at 270°C. 1 µL was injected at split mode with split ratio of 1:10. Calibration curves were linear between the concentration ranges 1000 – 5000 ppm. The method was validated for specificity, linearity, precision, accuracy, limit of quantitation and stability. This method can be directly used for the quantification of NIS as bulk drug and it is also suggested to use for pharmaceutical preparation.

Keyword: N-Iodosuccinimide; assay; GC-FID; chromatography; validation

INTRODUCTION

N-Iodosuccinimide (NIS) [Fig 1] is an important N-halo compound, which can be used for mild oxidation and iodination [1]. As part of our research, oxidation of various amino acids by NIS in aqueous acetic acid medium has been investigated [2,3]. Currently the assay for NIS can be estimated based on conventional titrimetric methods. Also extensive literature survey reveals that no chromatography methods currently available for the determination NIS. Therefore, the purpose of this investigation was to develop and validate a method using a simple, rapid, sensitive, precise, accurate and specific gas chromatography (GC) method.

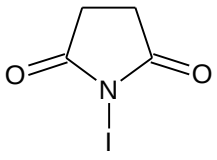

Fig. 1: Structure of N-Iodosuccinimide (NIS)

MATERIALS AND METHODS

N-Iodosuccinimide (Merck), Methanol (Merck), Shimadzu Gas chromatography 2010 plus equipped with auto sampler and Flame ionization detector (GC-FID), HP5 column 30mtX0.32 ID, Film thickness 0.25 micron. Injector and detector temperature is 260 and 270°C respectively. Column temperature gradient program of 110 to 240°C. 1µL was injected in split mode, with column flow 1.5 uL, Split ratio 1:10. The carry gas Nitrogen (N2) was kept constant throughout the analysis. Hydrogen (40 ml/min) and synthetic air (400ml/min) were used as auxiliary gases for the flame ionization detector (FID).

Sample preparation

For this analysis various concentration solutions of NIS, viz 1000, 2000, 3000, 4000 and 5000 ppm solutions was made using methanol as solvent. For solvent selection, precision and accuracy study 1500 ppm solution in methanol and acetone was prepared. [9] All these solutions are sonicated at room temperature for 15 min, kept under dark conditions and used amber container, vials to avoid light exposure. All these solutions are filtered through 0.45 micron nylon filters before injection.

Method development and optimization

The method development for the assay determination was based on its chemical properties. NIS is a polar molecule and therefore a polar solvent methanol was used as diluent.[2] The capillary column coated with 5% Phenyl and 95% dimethypoly-siloxane is a good choice for the separation of the analyte (HP5 column), since it provides symmetrical peaks with good resolution. Also these HP5 columns are widely used in most applications and affordable. The GC-FID parameters used in the method development were based on the better elution conditions of peaks. [7] The injection port and detector temperature were set as 260°C and 270°C respectively. Different temperature programs are investigated for GC oven. At the end of this study, the optimum temperature program was selected for a good resolution. The temperature programs of the GC oven with a run time of 12 min was as follows: Initial temperature 110°C hold for 4 min, finally increased to 240°C at the rate of $10°C^{-1}$ min and held for 3min. The total run time would 12min. The head pressure was set to ensure a hydrogen flow 40 ml^{-1}min. Split mode was chosen. The solvent, column and acquisition parameter were chosen to be starting point for the method development.

The limit of detection (LOD) and limit of quantitation (LOQ) were evaluated by series dilutions of NIS stock solutions in order to obtain signal to noise ratio of 3:1 for LOD and 10:1 for LOQ.

Stability study purpose, concentration of 5000 and 1000 ppm were prepared and was injected in to GC system at different time intervals. [7,8]

RESULTS AND DISCUSSION

The retention time of NIS would be approximately on 4.19 min with good peak shape. No further optimization of the method was required in terms retention time. Additionally, preliminary precision and linearity studies performed during the development of the method [4] showed that the 1 µL injection volume was reproducible and peak response was significant at the analytical concentration chosen. Typical chromatogram obtained with NIS at retention time 4.19 min is presented in [Fig 2].

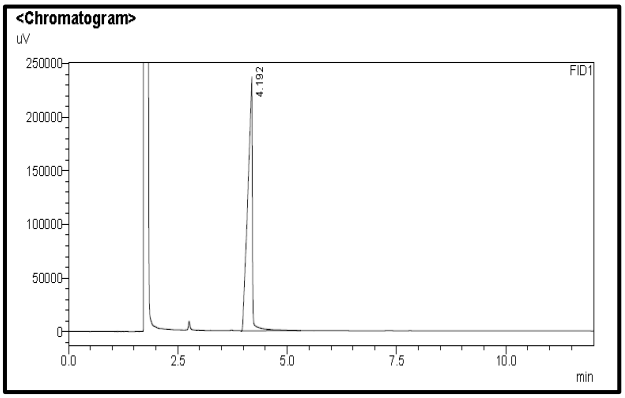

Fig. 2: GC-FID Chromatogram of N-Iodosuccinimide (NIS) 5000 ppm

Method Validation

Solvent Selection

Solubility of NIS has been tested with various solvents, NIS shows good solubility with methanol and acetone. Hence further study has been conducted to choose better and compactable one. For this study purpose 1500 ppm of NIS in methanol and acetone was prepared and injected in the same instrument conditions. The area response for methanol is higher than the area response for the NIS dissolved in acetone. Also peak shape was better with methanol (TF ≤1.18) compare to acetone (TF ≤1.52). Based on the results methanol been used as solvent for this study.

Precision and Accuracy

The precision of the analytical method was determined by repeatability, multiple injection of the same sample concentration were analysed six times in a day for precision [5,7]. The RSD value for precision was 1.39%. Retention times are more consistent with RSD 0.05%. These values are summarized in [Fig 3] [Table 1].

Table 1. Precision of RT and Area response for NIS

[NIS] = 5000 ppm	Same day		between day	
	RT	Area	RT	Area
Mean	4.194	1764818	4.208	1844557
SD	0.001	0.014	0.0004	0.004
RSD%	0.05	1.39	0.04	0.43

Number of injection n=6; All values are average of six replicate determinations.
SD: Standard deviation of six replicate determinations, RSD: Relative standard deviation.

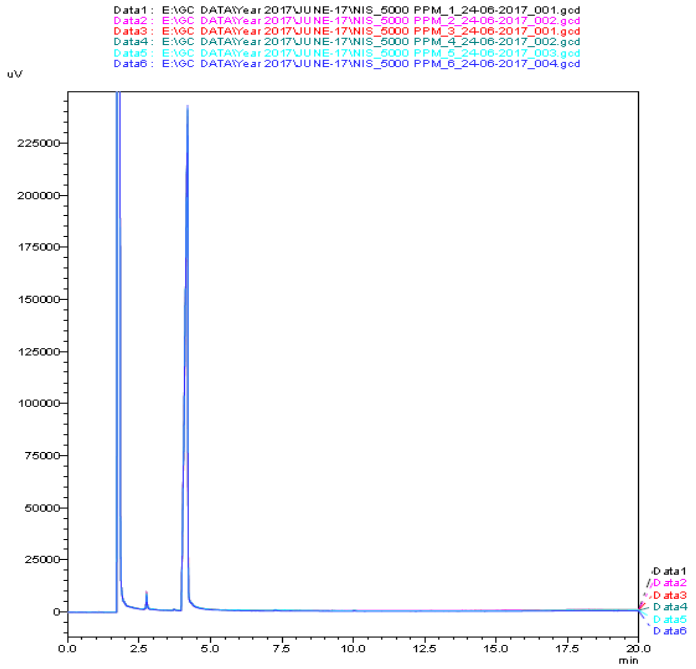

Fig. 3. Data comparison report of NIS 5000 ppm

Linearity

The linearity of peak area response versus concentration for NIS between concentration ranges of 1000 to 5000 ppm was studied. The calibration cure was drawn and evaluated by its correlation coefficient. The calibration equation from six replicate experiments, y= 356.82 x - 88293 (r^2= 0.9997) which demonstrated the linearity of the method [7][Fig 4].

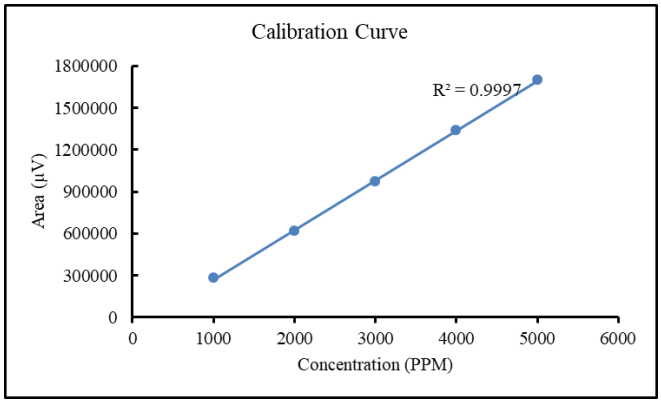

Fig. 4: Linearity calibration curve of NIS (1000 – 5000 ppm)

Sensitivity

The LOD and LOQ values for the analyte were found to be 100 ppm and 500 ppm respectively. Quantification determined for unknown concentration through the calibration curve has been shown in [Fig 5]. From the calibration curve, it is determined as 1516 ppm for 1500 ppm. The results has the relative standard deviation of 1.06%. Based on the area percentage NIS assay as bulk drug estimated as 98.2%. It is not suggested to analyse below 500 ppm, since NIS peak shape was broadening and tailing factor raises >2%. [7,8].

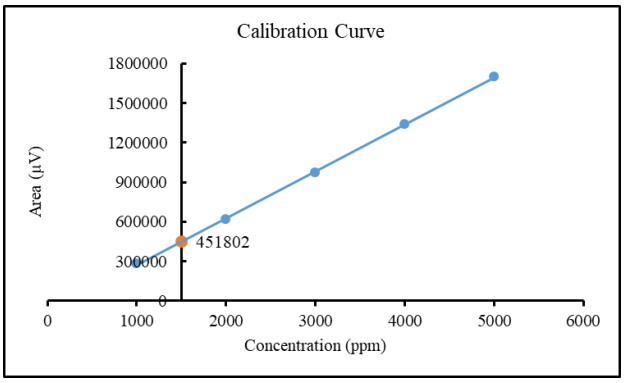

Fig. 5: Quantification model curve for NIS (1500 ppm)

Stability

Stability studies indicated that the samples were stable when kept at room temperature 27±2°C under dark condition for 72 hrs. The content of NIS as area percentage was determined at each interval [6], the sample solutions was stable over a period of 72 hours. Based on the diluent selectivity the stability of NIS also varies.

CONCLUSION

In the present study, a simple, rapid, reliable, sensitive, accurate and precise GC-FID method for the determination of NIS has been developed and validated. The method has produced satisfactory validation data for the tested parameters as per the ICH guidelines [7,8]. This GC-FID method is cost efficient as it uses commonly used HP5 capillary column, with moderate run time. The method proposed in this study has been effectively and efficiently used to analyze NIS during bulk drug manufacturing to estimate its assay and in formulations.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interests regarding the publication of this paper.

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