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DEVELOPMENT AND VALIDATION OF GAS CHROMATOGRAPHIC METHOD FOR ESTIMATION OF ISO-AMYL-2, CYANOACRYLATE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple and rapid analytical method for determination of iso-amyl-2, cyanoacrylate in pharmaceutical dosage form was developed and validated using gas chromatography (GC). The solutions of standard and the sample were prepared in methylene dichloride. GC separation was performed in about 3.649 min using a 30 m x 0.32 mm I.D. (film thickness 0.25 µm) HP-5 capillary column. Helium was used as carrier gas at a flow-rate of 1 ml min^{-1}. After injection of the sample at inlet temperature 230^0C, the temperature of the GC oven was as follows: initial temperature was 150^0C, held for 5min, increased to 200^0C at a rate of 50^0C min^{-1} held for 5 min, and finally to 240^0C at a rate of 5^0C min^{-1} and held for 3.3 min. Detector temperature is 250^0C. 2 µl was injected in splitless mode. Calibration curve was linear between the concentration range 50-150 µg/ml. The method was validated for specificity, linearity, precision, accuracy and limit of quantitation. Also, the method was applied to directly and easily to the analysis of the pharmaceutical dosage form.

Keywords: GC-FID, iso-amyl-2,cyano acrylate, HP-5 column.

INTRODUCTION

Iso-Amyl 2 Cyanoacrylate is an advanced gamma sterilized, non-pigmented, nontoxic, non-allergic, and biostatic tissue adhesive[1]. Chemically it belongs to the class of organic compounds known as cyanoacrylates. It helps in rapid wound closure with minimal scarring, and reduces the risk of postsurgical infection and trauma, apart from being simple to use, and showing a demonstrable safety[2], thus providing effective wound healing with minimal risk. The mechanism with which Iso-Amyl 2-cyanoacrylate acts is by getting converted into a polymer on coming in contact with moisture, and though, by itself, it is an inert material, it solidifies rapidly within 5-10 s. Chemical formula is $C_9H_{13}NO_2$ and chemical weight is 167.2 g/mol (Fig. 1: Chemical structure of Iso-Amyl-2-Cyanoacrylate).

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No previous GC-FID method for the determination and quantification of Iso-Amyl-2-Cyanoacrylate in pharmaceutical preparations in literature. Therefore, the purpose of this investigation was to develop and validate a simple, rapid, sensitive, precise, accurate and specific GC method.

MATERIALS AND METHODS

CHEMICALS AND REAGENTS

Iso-Amyl-2-Cyanoacrylatewas purchased from GSK Ltd., Mumbai,and other chemicals and solvents used were of analytical grade. Amcrylate injection containing 130 mg of Iso-Amyl-2-Cyanoacrylatewas obtained from Concord Drugs Ltd., Hyderabad.

Instrumentation

The GC-FID system was performed an Agilent 6890 N Network GC equipped with a flame ionization detector, Agilent 7683 series auto sampler, Agilent chemstation and HP-5 column with 0.25 µm film thickness (30 m x 0.320 mm I.D.). Injection and detector

temperature is 230 and 250^{0}C, respectively. 2 µl was injected in split less mode. The carry gas (He) flow-rate was kept constant during the run at 2 ml min^{-1}. Nitrogen (25 ml min^{-1}), hydrogen (40 ml min^{-1}) and synthetic air (400 ml min^{-1}) were used as auxiliary gases for the flame ionization detector.

Preparation of the standard solution

The stock standard solution of Iso-Amyl-2-Cyanoacrylatewas prepared by weigh 130mg drug transfer into 10ml of volumetric flask and dissolved in little amount of methylene dichloride (purity 99%)and make up the volume.

Preparation of the sample solution

Exactly take the sample of Iso-Amyl-2-Cyanoacrylate equivalent to 130mg and transfer into 10ml of volumetric flask and dissolved in little amount of methylene dichloride (purity 99%) and make up the volume.

RESULTS AND DISCUSSION

Method development and optimization

The method development for the assay of Iso-Amyl-2-Cyanoacrylate was based on its chemical properties. Iso-Amyl-2-Cyanoacrylate is a polar molecule and, therefore, a polar solvent methanol was used as the diluent. The capillary column coated with 5% phenyl, 95% dimethylpolysiloxane is a good choice for separationof this analyte since they elute as symmetrical peaks at a wide range of concentrations. The GC-FID parameters used in the method development were based on the boiling point. The injection port and detector temperature were set to 230 and 250^{0}C, respectively. Different temperature programs were investigated for GC oven. The temperature programs of the GC oven with a run time of 13 min was as follows: initial temperature 150^{0}C, held for 1.5 min, increased to 240^{0}C at a rate of 50^{0}C min-1 held for 5 min, and finally to 250^{0}C at a rate of 10^{0}C min^{-1} and held for 3.3 min. The head pressure was set to ensure a hydrogen flow of 40 ml min^{-1}. The split less mode was chosen. The solvent, column and acquisition parameters were chosen to be a starting point for the method development.

The retention time of Iso-Amyl-2-Cyanoacrylate was approximately 3.649 min with good peak shape. No further optimisation of the method was required. Additionally, preliminary precision and linearity studies performed during the development of the method showed that the 2 µl injection volume was reproducible and the peak response was significant at the analytical concentration chosen. Typical chromatogram obtained with standard Iso-Amyl-2-Cyanoacrylate is presented in Figure 2.

METHOD VALIDATION

Linearity

The linearity of peak area response versus concentration for Iso-Amyl-2-Cyanoacrylate was studied between concentration range of 50-150 µg/ml. The calibration curve constructed was evaluated by its correlation coefficient. The calibration equation from six replicate experiments, y = 415024x (r = 0.9997), demonstrated the linearity of the method Table 1 and figure 2.

Precision

The precision of the analytic method was determined by repeatability (within-day) and intermediate precision (between-day). The RSD value for within-day precision was ≤3.02% and for between-day precision was ≤3.82% Table 2 and 3.

Accuracy (Recovery)

To determine the accuracy of the proposed method and to study the interference of formulation additives, the recovery was checked as three different concentration levels (75, 100, 125 %w/w) and analytical recovery experiments were performed by adding known amount of pure drugs to pre-analyzed samples of commercial dosage form. The percent analytical recovery values were calculated by comparing concentration obtained from the spiked samples with actual added concentrations. These values are also listed in Table 4.

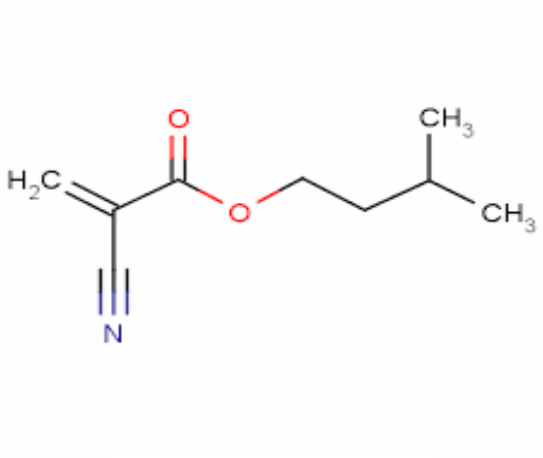

Fig. 1: Chemical structure of Iso-Amyl-2-Cyanoacrylate

Table 1: Linearity values of Iso-Amyl-2-Cyanoacrylate

Concentration (nm/ml)	Area			Mean
	Run 1	Run 2	Run 3	
50	20721180	20438219	20218854	20459418
75	31081769	31211432	32414362	31569188
100	41442359	41582240	41282843	41435814
125	51802949	52101199	51641276	51848475
150	62163539	62282489	62149632	62198553
Correlation coefficient				0.9997

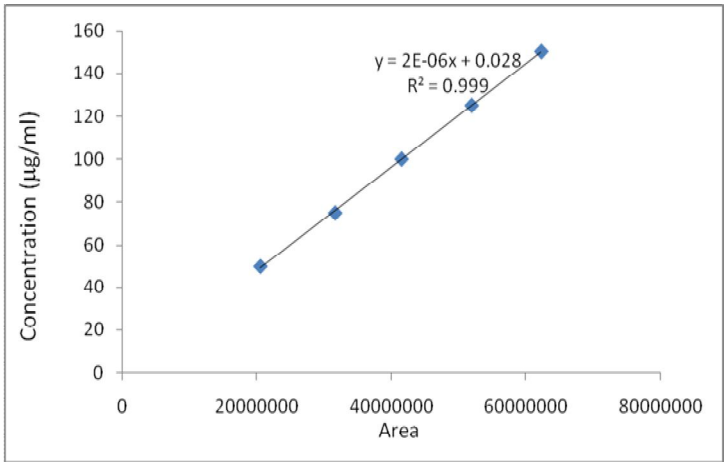

Fig. 2: Calibration curve of Iso-Amyl-2-Cyanoacrylate

Table 2: System precision

values of Iso-amyl-2, cyanoacrylate

Inj. No.	Area	RT
1	41511599	3.658
2	41242422	3.629
3	41443219	3.679
4	41202044	3.616
5	41342449	3.649
6	41494480	3.611
Mean	41372702	3.6403
SD	131230.47	0.0263186
% RSD	0.31719	0.7229712

Table 3: Method precision values of Iso-amyl-2, cyanoacrylate

Inj. No.	Area	RT
1	41411598	3.641
2	41554481	3.639
3	41342449	3.604
4	41292466	3.637
5	41763219	3.626
6	41142325	3.624
Mean	41417756	3.6285
SD	216936.53	0.0126853
% RSD	0.5237766	0.3496016

Table 4: Accuracy (% Recovery studies) values of Iso-amyl-2, cyanoacrylate

Sample spike (% w/w)	Area			Mean	Recovery (% w/w)
	Run 1	Run 2	Run 3		
75	27662774	25941996	26411993	26672254	89
100	40613512	40843296	40743481	40733430	98
125	53875067	52944981	53105019	53308356	104

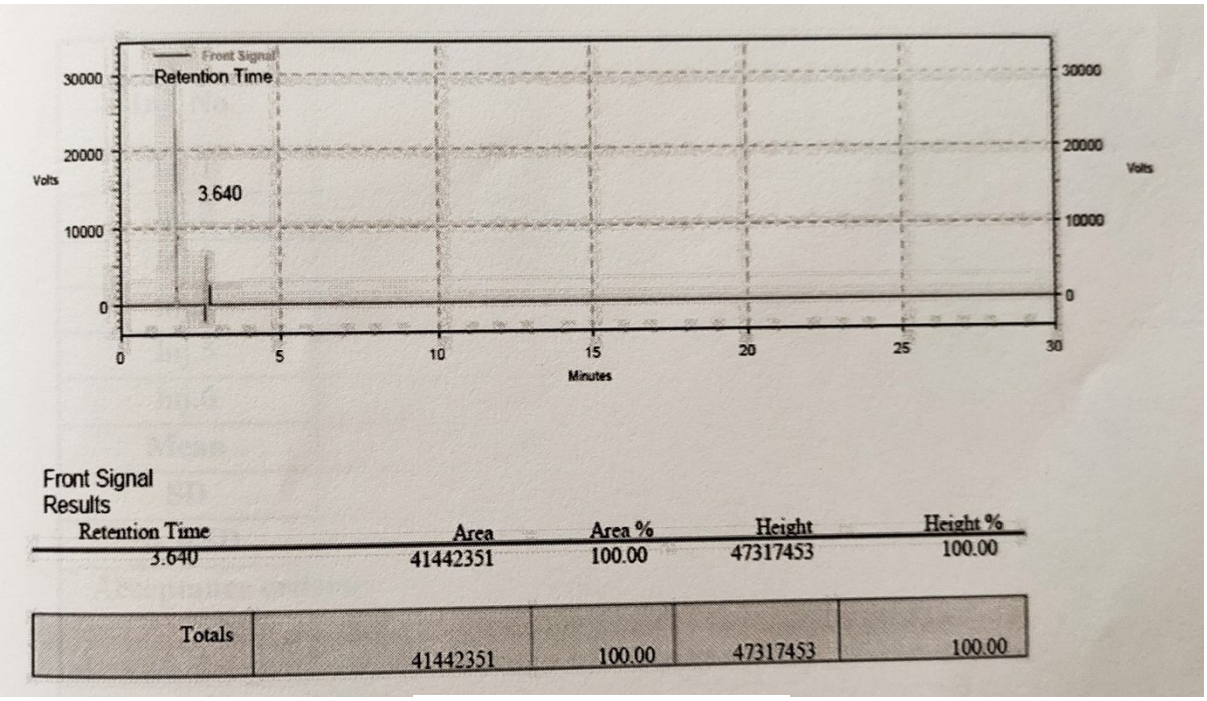

Fig. 3: Typical Chromatogram

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It is concluded that the method is robust as it is found that the % RSD is less than 2 concerning flow rate (1.1ml/min1.3ml/min), mobile phase ((-) 35B:65A-(+) 45B:55A) and temperature ((-) 25°C-(+) 35°C).

CONCLUSION

In the present report, a simple, rapid, sensitive, reliable, specific, accurate and precise GC-FID method for the determination of iso-amyl-2, cyanoacrylate in pharmaceutical preparation was developed and validated. The method described in the present report has been effectively and efficiently used to analyzeiso-amyl-2, cyanoacrylate pharmaceutical dosage form without any interference from the pharmaceutical excipients. Therefore, GC-FID method can be used for the routine QC analysis of iso-amyl-2, cyanoacrylate in pharmaceutical preparations.

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