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GC method for separation and quantification of positional isomers of trifluoro methoxy aniline and trifluoromethoxy nitro benzene in 4-(trifluoromethoxy aniline), a key intermediate of Riluzole

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ABSTRACT

A sensitive and simple GC method was developed for the separation and quantification of related impurities in 4-(trifluoromethoxy Aniline) (4-TFMA), which is a key intermediate of Riluzole. The analysis was performed by using FID detector and AT-210, 30 meters, 0.53 mm internal diameter and 1.0 µm GC capillary column with 3.0 psi pressure. In 4-TFMA, two more positional isomers like 3-TFMA and 2-TFMA were possible impurities, at the same time, its intermediate nitro compound also contains three positional isomers, like 2-nitro, 3-nitro and 4-nitro, also there is a possibility of two more intermediates like trifluoromethoxy benzene (TMB) and also its starting material anisole as an impurities. A single analytical method was developed for the separation of all theses impurities including six positional isomers with the quantitation and detection limits of less than 4µg mL^{-1} and 0.8µg mL^{-1} respectively. The Analytical method was validated as per ICH.

KEYWORDS

Gas chromatography;
Riluzole;
Analytical method validation;
Quantification;
Positional isomer.

INTRODUCTION

Riluzole (Figure 1), 2-amino-6-trifluoromethoxybenzothiazole, is an anti-glutamatergic agent, which was found to be protective in several models of neurodegenerative diseases including amyotrophic lateral sclerosis (ALS)[1], multiple sclerosis (MS)[2], Parkinson's disease (PD)[3], and ischemia[4]. Riluzole was demonstrated to modulate the anti-glutamatergic activity through glutamate and sodium receptors. Several studies showed that Riluzole inhibits the release of glutamate and l-aspartate from nerve terminals, modulates the N-methyl-d-aspartate (NMDA) ionotropic receptors and stabi-

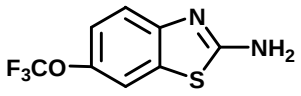

Figure 1 : Riluzole

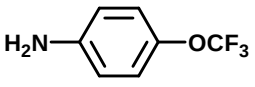

Figure 2 : 4-Trifluormethoxy aniline

Full Paper

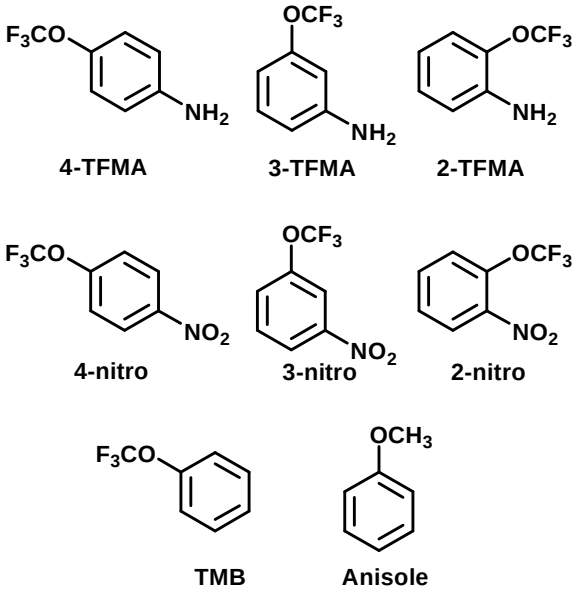

Figure 3 : 4-trilfuormethoxy aniline and its related compounds

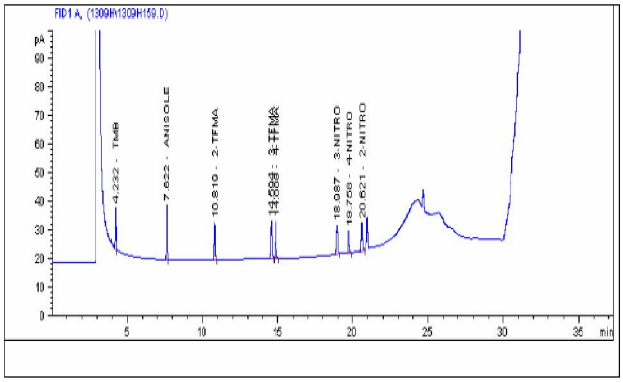

Figure 4 : Typical GC chromatogram of 4-TFMA and its related compounds

lizes the voltage-dependent sodium channels in myelinated fibers[5]. Due to its interesting pharmacology properties and potential therapeutic applications, a sensitive and simple analytical method for the quantification of impurities in its key intermediate, 4-(trifluoromethoxy aniline) (4-TFMA) (Figure 2) could be essential. As far as our knowledge, there is no analytical method to separate and quantify all these impurities in 4-TFMA. However, some of the analytical methods describes the determination of Riluzole in human plasma and urine by using HPLC[6] or coupled with tandem mass spectrometry[7].

Accordingly, the aim of the present study was to develop an analytical method that can be able to separate and quantify the relates substances like possible positional isomers of Trifluormethoxy aniline (3-TFMA and 2-TFMA), three positional isomers of Trifluormethoxy nitro benzene (4-nitro, 3-nitro and 2-nitro), Intermediate, Trifluorometoxy benzene and starting material, Anisole in 4-trifluormethoxy aniline. Hence, the presence of these positional isomers in key intermediate of Riluzole was carried over all the way to final and results in the formation of impurities like positional isomers of Drug substance. And also to validate the method as per ICH[8-10].

TABLE 1 : Sensitivity

S. No.	Name of the compound	Limit of quantitation (µg mL^{-1})	Limit of detection (µg mL^{-1})
1	4-TFMA	2.5	0.5
2	3-TFMA	2.5	0.5
3	2-TFMA	2.5	0.5
4	4-Nitro	4.0	0.8
5	3-Nitro	3.0	0.6
6	2-Nitro	3.5	0.7
7	TMB	1.5	0.3
8	Anisole	1.0	0.2

TABLE 2 : Robustness

S.No.	Parameter	Variation	Resolution between 4-TFMA & 3-TFMA
1	Flow	3.0 psi (As such)	2.06
		2.8 psi	2.06
		3.2 psi	2.03
2	Column oven temperature	50°C (As such)	2.06
		45°C	2.05
		55°C	2.01

EXPERIMENTAL

Chemicals

Samples of 4-TFMA its impurities namely, 2-TFMA, 3-TFMA, 2-nitro, 3-nitro, 4-nitro, TMB and Anisole from Sigma-Aldrich. The structures and chemical names of impurities, shown in figure 3. Chromatography grade methanol from Merck, Darmstadt, Germany.

Instrumentation

The GC system, used for method development and validation of the developed analytical method was Agilent Technologies 6890N Network GC system with 7386 series auto sampler. The output signal was moni-

tored and processed using Chemstation software on Pentium Computer (Digital Equipment Co).

Chromatographic conditions

The chromatographic column used was AT-210, 30 meters, 0.53 mm internal diameter and 1.0 μm from LCGC. Helium was used as carrier gas at a constant flow of 3.0psi. A flame ionization detector (FID) was used for detection of impurities. The Hydrogen, air and makeup flows were kept at 40, 450 and 30 mL min^{-1}, respectively. The detector temperature was set at 260 °C. The GC column oven was programmed as initially 50°C, then raised to 125°C at the rate of 3°C per minute, and hold at 125°C for 5 minutes and then, railed to 230°C at the rate of 45°C per minute and hold at 230°C for 5 minutes. The inlet temperature was kept at 200°C. The samples were injected in a split mode 1:5 with a 1.0 μL of injection volume.

Preparation of solutions

A solution of 4-TFMA (50 mg mL^{-1}) was prepared by dissolving appropriate amount in the diluent. A stock solution of impurities (mixture of 2-TFMA, 3-TFMA, 2-nitro, 3-nitro, 4-nitro, TMB and Anisole) were prepared at concentration of 500 μg mL^{-1}.

RESULTS AND DISCUSSION

Method development and Optimization of Chromatographic conditions

The primary target of this work was to develop an analytical method, which could separate all the above mentioned 7 impurities including six positional isomers in single method and it can be quantifiable at lower levels. Initially, tried with HPLC methods, using UV detector found that the wavelength maxima for some of the impurities and starting material were around 200 nm. So, Baseline noise was more at lower wavelength ranges and to get optimum limit of quantification and detection, we switched over to GC technique for development.

The optimized chromatographic conditions by using Gas Chromatograph were, AT-210, 30 meters, 0.53 mm internal diameter and 1.0 μm from LC-GC. Helium was used as carrier gas at a constant flow of 3.0psi. A flame ionization detector (FID) was used for detection of impurities. The GC column oven was programmed as initially 50°C, then raised to 125°C at the rate of 3°C per minute, and hold at 125°C for 5 minutes and then, railed to 230°C at the rate of 45°C per minute and hold at 230°C for 5 minutes. The inlet temperature was kept at 200°C.

In the above optimized chromatographic conditions of Gas chromatography 4-TFMA and it's related 7 impurities well resolved with a resolution of more than 2.0 and the typical retention times of 4-TFMA, 2-TFMA, 3-TFMA, 2-nitro, 3-nitro, 4-nitro, TMB and starting material Anisole were 14.9, 10.8, 14.6, 20.6, 19.0, 19.8, 4.2 and 7.6 minutes respectively. (Figure 4).

Sensitivity

The lowest LOD and the LOQ were determined based on signal-to-noise ratios using analytical responses of 3 and 10 times the background noise, respectively The Limit of detection and quantification for all the 7 impurities along with 4-TFMA were given in TABLE 1.

Linearity

To establish the Linearity of the method, calibration solutions were prepared from the stock solution of 7 impurities, concentration ranges from LOQ to 1125 μg mL^{-1}. The correlation coefficient, slope and Y-intercept of the calibration curve were calculated.

Precision

The repeatability (intra-day) and the intermediate precision (inter-day) of the method was evaluated by the determination of peak area percentage RSD of all 7 impurities for six replicate injections of spiked sample at the levels of LOQ and 750 μg mL^{-1}. The intermediate precision (Ruggedness) of the method was evaluated by different analyst using different column and different instrument in the same laboratory.

Accuracy

Accuracy of the method was demonstrated at the four different concentration levels in triplicate. The analysis was carried out at the concentration levels of LOQ, 375 μg mL^{-1}, 750 μg mL^{-1} and 1125 μg mL^{-1}. The percentage recoveries were in between 96 and 104.

Robustness

The Robustness of the method was studied by varying number of method parameter. The experimental con-

Full Paper

ditions were deliberately varied in order to determine the impact on resolution of critical pair of 4-TFMA and 3-TFMA. The flow rate on resolution was studied by varying the flow rate by±0.2 psi. The temperature effect was studies by varying initial temperature of column oven ±5°C. No significant change observed in the resolution between the critical pair. Details of the results obtained are listed in TABLE 2.

CONCLUSION

A sensitive, simple, precise, accurate and robust GC method was developed and established the Limit of quantification and Limit of detection of all the impurities. And also validated the developed method as per ICH. Hence, this method can be used in quality control laboratories for the routine analysis.

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