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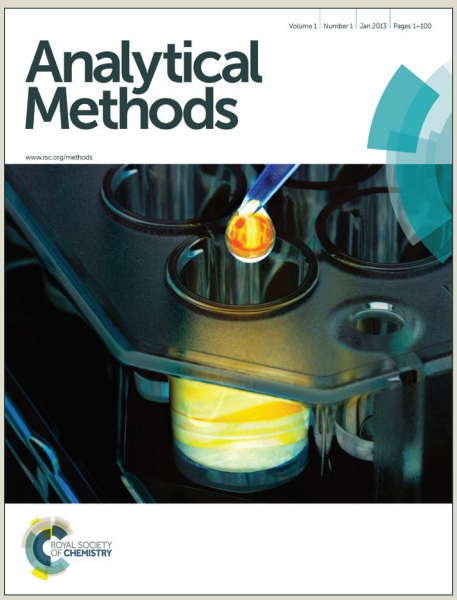

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Rapid and highly efficient gas chromatographic method for the separation and determination of bromofluorobenzaldehydes with the application of low thermal mass technology (LTM)

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ABSTRACT

A sensitive, rapid and highly efficient low thermal mass (LTM GC) method has been developed for the separation and quantitative determination of 10 isomers of bromofluoro benzaldehyde. LTM GC is a new approach for modern high speed GC and the technology is to achieve ultrafast temperature programming with an unprecedented cool down time and a power consumption of approximately 1% of conventional GC. The chromatographic separation was achieved using DB-624 column with ultrafast temperature programming. The method was validated according to ICH guidelines with respect to specificity, precision, linearity and accuracy. Regression analysis showed correlation coefficient value greater than 0.999 for all isomers. Detection limit and quantitation limit established for each isomer was 0.4ppm and 1.2ppm respectively indicating high sensitivity of the newly developed LTM GC method. Accuracy of the method was established based on the recovery obtained between 93.7% and 107.7% for all isomers.

Keywords: Bromofluoro benzaldehyde; Starting material; Isomers; LTM GC; Validation.

Introduction

Impurity control in pharmaceutical products is a primary goal of drug development. Controlling the quality of starting materials used to prepare active pharmaceutical ingredients (APIs) is a critical part of ensuring the ultimate quality of the APIs.[1] This is particularly important in terms of impurities that originate with the starting material and can carry through directly or participate in the reaction chemistry to produce significant impurities in the APIs.[2-3] An API key starting material is defined as a compound used in the synthetic sequence that contains a significant structural fragment of the APIs.[1] Impurities in a starting material are likely to be structurally related to the starting material and this is very challenging to detect or separate these impurities in any of the analytical technique especially when isomers are present in the starting material.[4] It is possible and often likely that different synthetic routes may be used by different suppliers to produce the starting material and these routes may produce different impurities.

Isomers of bromofluoro benzaldehyde are widely used in research work especially in drug synthesis and preparation of intermediates. The reason may be due to the presence of highly active functional groups (halides and aldehyde groups) and it can be used for the preparation of variety of compounds. The common reaction of aryl halides are Substitutions reaction, Eliminations reaction, Grignard reaction, Suzuki reaction, Rosenmund-von Braun reaction, Finkelstein reaction, Ullmann reaction etc. Aldehyde group also highly reactive and the common reactions are Reduction, Oxidation, Bisulphite reaction, Wittig reaction, Pinacol coupling reaction, Wolff-Kishner reduction, Johnson-Corey-Chaykovsky reaction etc. Therefore when we perform such reactions, quality attributes of starting material is very crucial. Evaluation of the different synthetic routes of bromofluoro benzaldehyde reveals that 10 isomers can be present in it and these isomers are left uncontrolled, they can participate in the reaction and carry forward to

subsequent stages. In pharmaceutical industry, isomers are important and an analytical method should be capable of separating all isomers. Extensive literature reveals that no analytical methods were reported for detecting or quantifying the isomers of bromofluoro benzaldehyde. Therefore, we felt the need to develop a method for separating all these isomers. The main objective of the study was to develop single, fast and accurate method for the separation and quantification of isomers of bromofluoro benzaldehyde. In order to accomplish this objective, we have utilized a new technology in GC called low thermal mass GC (LTMGC).

In GC, the process of increasing column temperature during analysis is referred to as temperature programming (TPGC) and it is often considered to be the second most important parameter to control, the first being column selectivity.[5] For a particular solute, TPGC leads to a decrease in retention volume and retention factor. The benefits of TPGC include better separation for solutes with a wide boiling range, improved detection limit, and improved peak symmetry, especially for solutes with high retention factors.[6-8] A new, recently introduced instrument incorporates technology to achieve ultrafast temperature programming with an unprecedented cool down time and a power consumption of approximately 1% of conventional GC. The technology is referred to as low thermal mass GC (LTM GC).[9-13] This article describes the development of single method for the separation of 10 isomers of bromofluoro benzaldehyde using LTM GC with very short run time. It is a new approach for modern high-speed GC.

Results and Discussion

In general terms, gas chromatography relies less on selectivity than HPLC to generate separation due to its higher inherent efficiency. Three parameters are typically adjusted to optimise a separation; column temperature and stationary phase type are used to optimise

selectivity whilst column dimensions are used to optimise efficiency. The selection of correct stationary phase is one of the most critical parameters in the success of any GC method. We have used three different stationary phases like DB-1 (100% dimethyl polysiloxane), DB-5 (5% diphenyl - 95% dimethyl polysiloxane) and DB-624 (6% cyano propyl - 94% dimethyl polysiloxane) having dimensions 20 m length × 0.18 mm ID with 1.0 μm particle size. Preliminary experiments were carried out by using non polar stationary phases like DB-1 and DB-5 for the separation of each isomer. Several temperature programmes were attempted with these columns however it was failed to separate all the isomers. Use of DB-624 as stationary phase was significant in achieving the desired resolution between isomers. At last with slow and ultra fast heating rate, all isomers were eluted and separated within the stipulated run time and the finalised method is described in section 2.2.

As a general rule of thumb, if the boiling point of two compounds differs by 30 ^{0}C or more then they may be separated by most stationary phases. This is due to the fact that dispersion is the dominant interaction for a wide range of stationary phases.[5] Boiling point of each isomer of bromofluoro benzaldehyde is close to each other and therefore, separation more possible by degree of interaction between stationary phase and isomers. Melting point and boiling point of each isomer is mentioned in the Table 2. The differences in dipole moment between the isomers are very small and using a non dipole interaction phase (DB-1 & DB-5), there is no discernible separations between these isomers were observed. However, when a dipole component is introduced into the stationary phase (cyano propyl), the separation begins to occur but with longer run time.

As temperature is convenient to influence within a GC experiment, and as it has a large effect of the selectivity of a separation, it is primary variable in method development and optimisation. According to Clapeyron-Clausius equation, when temperature is decreased,

analyte vapour pressure decreases and hence retention time increases. The experiment started with a gradient temperature programme in which initial column temperature was set at 50 ^{0}C and gradually increased to 160 ^{0}C at heating rate of 10 ^{0}C per minute, hold for 2 minutes and then increased to 260 ^{0}C at heating rate of 100 ^{0}C per minute. After some trial with the temperature programmes it was realised that at 100 ^{0}C temperature, all isomers were initially immobilised at the head of the column, where they remain until the column temperature is suitable for them to begin to vaporise and partition through the column. However it was observed that the peak elution was not satisfactory and therefore decided to change initial temperature to 140 ^{0}C and hold for 2 minute to save time. In order to prevent co-elution of early eluting peaks, heating rate was maintained as low as 5 ^{0}C per minute initially and lowering the initial hold time facilitated to gets better resolution between early eluting peaks and improved peak shape without increasing analysis time. Then it was noticed that increasing the ramp rate has the effect of reducing resolution between the mid eluting peaks and dropping the overall analysis time. Ultimately reducing the ramp rate resulted increasing the resolution between mid eluting isomers but increases overall analysis time. In order to reduce the analysis time, multiple ramp rates used at a rate of 270 ^{0}C per minute after 3.6 minute. The last resort in developing good resolution was the use of a mid ramp hold. Here an isocratic portion is inserted into the gradient to increase resolution between isomer-8 and isomer-9 and this approach was very effective in achieving adequate baseline separation between two isomers. The final temperature was maintained at 260 ^{0}C and hold time 1 minute to ensure that all late eluting unknown peaks with higher boiling point are eluted within the stipulated time. At the end of this investigation, the best temperature program was selected for a good resolution, good peak shape and short run time is described in section 2.2. The total run time of the method was 5 minute with a resolution more than 1.2 for

each isomer. Spiked chromatogram of 10 isomers of bromofluoro benzaldehyde is depicted in Fig.1. All isomers are listed in Table 1 and labelled based on their elution pattern. Later, the same approach has been investigated using conventional GC. However long run time, poor peak shape and poor resolution were observed compared to LTM GC method. Therefore it was concluded from this study is that LTM GC was very helpful in developing a method for separating the isomers of bromofluoro benzaldehyde with very short run time.

Method validation

The newly developed LTM GC method was validated for sensitivity, linearity, precision and accuracy, robustness and system suitability according to ICH guidelines.[14] Validation study was carried out for all isomers. The system suitability and selectivity were checked by injecting 40 ppm of all isomers mixture monitored throughout the validation. Method validation results are summarized in Table 3.

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

The limit of detection and limit of quantitation were determined for all isomers of bromofluoro benzaldehyde as per ICH $Q2R_1$ guideline. The LOD and LOQ for each isomer were estimated at a signal-to-noise ratio of 3:1 and 10:1, respectively by injecting a series of diluted solutions with known concentration. The limit of detection and the limit of quantitation for all isomers were about 0.4 ppm and 1.2 ppm respectively. The calculated LOQ concentrations of all the components were verified for precision by injecting six individual preparations of isomers. The RSD of LOQ precision was in the range of 2.9 to 4.2 %.

Linearity and range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of the analyte in the sample. A linearity test solution for the isomers was prepared by diluting the isomers stock solution to the required concentrations. The solutions were prepared at six concentration levels from LOQ to 400ppm (i.e. LOQ, 4ppm, 40ppm, 100ppm, 160ppm and 400ppm) was subjected to linear regression analysis with the least squares method. Calibration equation obtained from regression analysis was used to calculate the corresponding predicted responses. The residuals and sum of the residual squares were calculated from the predicted responses. The correlation coefficient obtained was greater than 0.999 for all impurities. The result showed an excellent correlation between the peak and concentration of all impurities. The range of the method was from LOQ to 400ppm for the each isomer.

Precision

Precision of the method was studied for method precision and intermediate precision. Method precision was checked by injecting six individual preparations of 2-Bromo-6-fluorobenzaldehyde (10000ppm) spiked with 20ppm of each other isomers. In the intermediate precision study, the similar procedure of method precision was carried out by a different day. % RSD of areas of each impurity was within 5.0, confirming good precision at low levels of the developed analytical method.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found. The accuracy of the method was evaluated in triplicate by spiking each isomer at the concentration of LOQ, 10ppm, 20ppm and 30ppm with 2-bromo-6-fluorobenzaldehyde (10000ppm). The percentage recovery of all impurities in drug substance has been calculated. Chromatogram of 2-Bromo-6-fluorobenzaldehyde spiked with other 9 isomers was depicted in Fig.2.

Robustness

To evaluate the robustness of the developed method, the chromatographic conditions were deliberately altered and the resolution between each peaks were evaluated. Close observation of analysis results of deliberately changed chromatographic conditions viz; flow rate (2.0±0.20 mL/min), heating rate (5±1 ^{0}C) and initial column temperature (140±5 ^{0}C) revealed that resolution between each peaks were greater than 1.2 and there was no significant change in the retention time for all isomers illustrating the robustness of the method.

Experimental

Materials and reagents

Isomers of bromofluoro benzaldehyde were procured from Sigma Aldrich, Bangalore, India. GC grade acetonitrile was purchased from Rankem, Mumbai, India.

Low Thermal Mass Gas Chromatography (LTM GC)

Samples were analysed on Agilent 7890A LTM GC equipped with 7693 autosampler and FID detector (Agilent technologies, CA, USA). The chromatographic separations were achieved on Agilent DB-624 column (20 mm length × 0.18 mm ID with 1.0 µm particle size). The carrier gas used was hydrogen with a flow rate 2.0 mL per minute and a flame ionization detector operated with 30 mL/min hydrogen and 350 mL/min air. The LTM GC temperature profile was 140 ^{0}C held for 1.0 min, then increased 5 ^{0}C /min to 150 ^{0}C, held for 0.6 min, then increased 270 ^{0}C /min to 260 ^{0}C, held for 1.0 min. The host oven temperature was maintained at 250 ^{0}C. The injector was operated in split mode (25:1) and the injector temperature was 240 ^{0}C. The detector temperature was 280 ^{0}C, and the injection volume was 1 µL.

Preparation of stock solutions for method validation

A test preparation of 10000 µg/mL solution of isomers were prepared by dissolving 100 mg of each isomer in acetonitrile and made up to 10 mL with acetonitrile. Transferred 1 mL of each individual stock solution into a 25 mL volumetric flask and made up to volume with acetonitrile.

Conclusion

A GC method is developed for the separation and quantification of bromofluoro benzaldehydes with the use of LTM technology. The benefit of LTM technology involves higher heating rate and cooling rate hence faster analysis. Different temperature programmes were studied using stationary phases like DB-1, DB-5 and DB-624. However satisfactory resolution between isomers was achieved with DB-624 column. The isomers were separated with the principle of classical GC conditions however ultra

fast heating with LTM technology resulted less analysis time. This newly developed method has been validated as per regulatory requirements and can be used for routine analysis.

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Table 1

Isomers of bromofluoro benzaldehyde

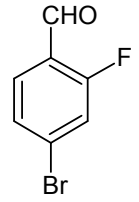
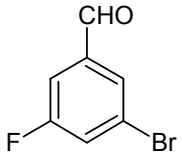
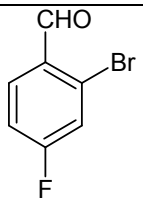
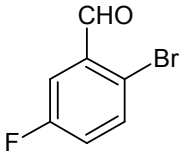
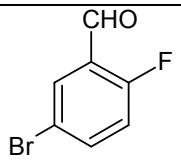
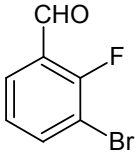
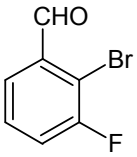
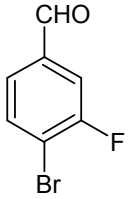
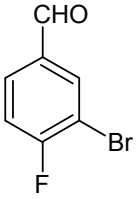
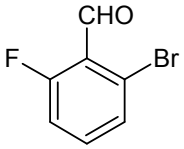
 4-Bromo-2-fluorobenzaldehyde Isomer-1	 3-Bromo-5-fluorobenzaldehyde Isomer-2
 2-Bromo-4-fluorobenzaldehyde Isomer-3	 2-Bromo-5-fluorobenzaldehyde Isomer-4
 5-Bromo-2-fluorobenzaldehyde Isomer-5	 3-Bromo-2-fluorobenzaldehyde Isomer-6
 2-Bromo-3-fluorobenzaldehyde Isomer-7	 4-Bromo-3-fluorobenzaldehyde Isomer-8
 3-Bromo-4-fluorobenzaldehyde Isomer-9	 2-Bromo-6-fluorobenzaldehyde Isomer-10

Table 2

Melting and boiling point of isomers of bromofluoro benzaldehyde

Isomer	Melting point (^{0}C)	Boiling point (^{0}C)
Isomer-1	58-62	241.4
Isomer-2	41-43	231.1
Isomer-3	61-63	234.9
Isomer-4	51-56	225.8
Isomer-5	58-62	230.1
Isomer-6	43-46	235.4
Isomer-7	43-46	227.4
Isomer-8	55-59	240.2
Isomer-9	21-33	237.8
Isomer-10	45-48	228.5

Table 3
Method validation summary report

Parameter	Isomer-1	Isomer-2	Isomer-3	Isomer-4	Isomer-5	Isomer-6	Isomer-7	Isomer-8	Isomer-9	Isomer-10
System suitability										
RT	2.404	2.476	2.524	2.581	2.726	2.905	2.952	3.041	3.116	3.617
Rs	-	2.07	1.35	1.60	3.96	4.70	1.21	2.25	1.85	11.32
N	78699	78654	80003	82383	86368	89662	89963	94112	93361	92421
T	1.05	1.08	1.08	1.12	1.10	1/08	1.06	1.07	1.06	1.19
Linearity										
r	0.9998	0.9997	0.9999	1.0000	0.9998	0.9999	0.9997	0.9999	0.9998	0.9999
Slope	0.4496	0.4894	0.5352	0.5210	0.4927	0.5300	0.4952	0.5368	0.4705	0.5214
Intercept	-1.1525	-0.1472	-0.2867	-0.2680	-0.2495	0.3550	-0.3446	-0.3227	-0.2946	-0.4670
Detection limit (ppm)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Quantitation limit (ppm)	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Precision (QL)	3.9	4.2	3.8	4.0	3.4	3.6	4.1	2.9	3.2	3.1
% RSD (n 6)										
Repeatability (intra day)	1.2	1.1	1.0	1.2	1.1	1.0	1.4	1.9	1.4	1.0
% RSD (n 12)										
Intermediate precision (inter day)	2.5	2.0	1.6	1.4	1.5	1.5	1.5	1.8	1.6	1.5
% RSD (n 12)										
Accuracy at QL level (n 3)										
Amount added (ppm)	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Amount recovered (ppm)	1.25	1.15	1.14	1.24	1.21	1.13	1.14	1.25	1.27	1.22
% Recovery	104.2	95.8	95.0	103.3	100.8	94.2	95.0	104.2	105.8	101.7
Accuracy at 50% level (n 3)										
Amount added (ppm)	10	10	10	10	10	10	10	10	10	10
Amount recovered (ppm)	9.37	9.42	9.53	10.43	9.98	9.43	9.95	10.22	10.43	10.72
% Recovery	93.7	94.2	95.3	104.3	99.8	94.3	99.5	102.2	104.3	107.2
Accuracy at 100% level (n 3)										
Amount added (ppm)	20	20	20	20	20	20	20	20	20	20
Amount recovered (ppm)	21.22	18.97	19.02	20.65	20.32	18.93	18.75	21.33	20.88	20.47
% Recovery	106.1	94.9	95.1	103.3	101.6	94.7	93.8	106.7	104.4	102.4
Accuracy at 150% level (n 3)										
Amount added (ppm)	30	30	30	30	30	30	30	30	30	30
Amount recovered (ppm)	31.12	28.82	28.78	30.94	30.56	28.10	28.25	31.22	31.03	30.12
% Recovery	103.7	96.1	95.9	103.1	101.9	93.6	94.2	104.1	103.4	100.4

n, number of determinations; RT, retention time; RRT, relative retention time; Rs , USP resolution; N, number of theoretical plates; T, USP tailing factor; r, correlation coefficient.

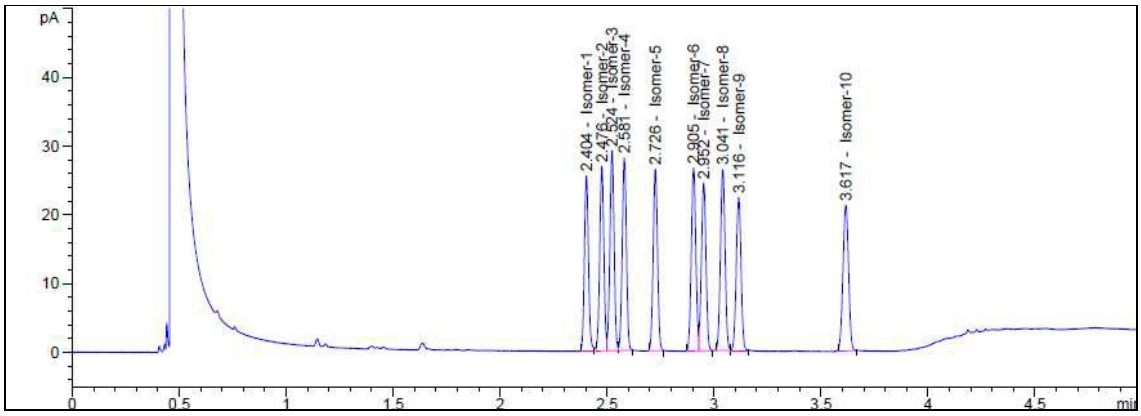


Fig. 1. Spiked chromatogram of 10 isomers of bromofluoro benzaldehyde.

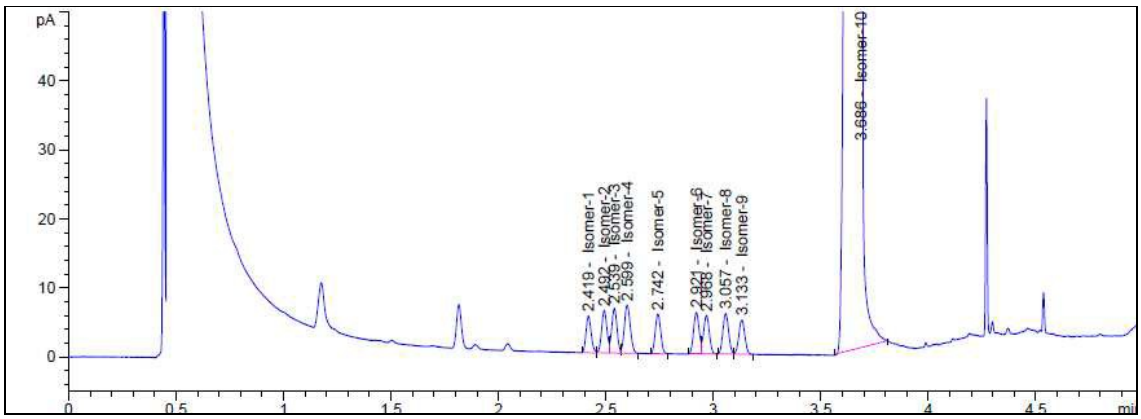


Fig.2. Chromatogram of 2-Bromo-6-fluorobenzaldehyde spiked with other 9 isomers.

Separation of 10 regio isomers of bromofluoro benzaldehyde by low thermal mass gas chromatography (LTM GC)