

Development of a Method for Regioisomer Impurity Detection and Quantitation within the Raw Material 3-Chloro-5-Fluorophenol by Gas Chromatography

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Abstract

Control of regioisomer impurities within pharmaceutical raw materials, intermediates, and active pharmaceutical ingredients are of major concern for the pharmaceutical industry. If regioisomer impurities are possible, their detection and quantitation should be established as early as possible within the process. This work describes a gas chromatography area% method for the detection and quantitation of all regioisomer impurities associated with 3-chloro-5-fluorophenol, a raw material used within the pharmaceutical industry along with other related impurities found within 3-chloro-5-fluorophenol. Several method development aspects, as well as a general regioisomer impurity control strategy related to 3-chloro-5-fluorophenol are discussed.

Keywords: Gas chromatography; Regioisomer; API; Raw material

Introduction

Related compounds (impurities) are always of concern when it comes to quality of raw materials and intermediates used for the production of an active pharmaceutical ingredient (API) [1-3]. One subclass of related compounds which tends to be more challenging to detect and quantify are regioisomer impurities (also known as positional isomers) [4]. Figure 1 shows two sets of regioisomer impurity examples for 1-propanol and *para*-anisidine.

A considerable amount of investment must be committed to ensure appropriate analytical methods are developed to ensure these impurities are properly controlled in raw materials, intermediates, and active pharmaceutical ingredients. Tactically, the earlier regioisomer impurities are controlled to acceptable levels within the process; analysis of further downstream intermediates and ultimately the API become simplified to typical related impurities (non-regioisomer impurities). Therefore, control of regioisomer impurities should take place within raw materials or when these impurities are found to be formed within a given process [5].

The raw material 3-chloro-5-fluorophenol poses a unique separation challenge since all three substituents are not the same functional group. Therefore, there are nine total regioisomer impurities possible within this raw material (Figure 2). Since 3-chloro-5-fluorophenol has the potential of being a raw material used in the synthesis of an API, development of an analytical method to ensure proper detection and quantitation of all possible regioisomer impurities was deemed to be an important research objective.

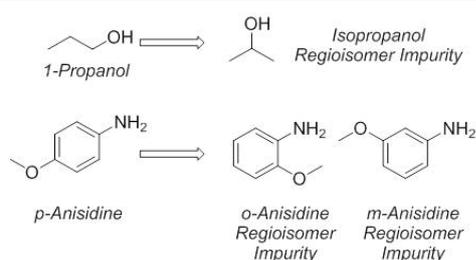


Figure 1: Regioisomer Impurity Examples.

Experimental

Chemicals and reagents

The following chemicals and reagents were utilized in this communication: Methanol (MeOH) Fisher Chemical Lot#161609; Acetonitrile (MeCN) Fisher Chemical Lot#162576; N,N-Dimethylacetamide (DMAc) Sigma-Aldrich Lot#SHBG3632V; N-Methyl-2-pyrrolidone (NMP) Acros Organics Lot#1338572; 2-Chloro-5-fluorophenol Acros Organics Lot#A020210301; 2-Chloro-3-fluorophenol Matrix Scientific Lot#N17P; 2-Chloro-4-fluorophenol Acros Organics Lot#A010381301; 2-Chloro-6-fluorophenol Aldrich Lot# 04724KH; 3-Chloro-4-fluorophenol Aldrich Lot#00419CS; 4-Chloro-2-fluorophenol Aldrich Lot#05418PU; 5-Chloro-2-fluorophenol BePharm Limited Lot#WZG091010-001; 3-Chloro-2-fluorophenol Matrix Scientific Lot#0091; 4-Chloro-3-fluorophenol Acros Organics Lot#A018902701; Phenol Sigma-Aldrich Lot#BCBK8781V, 3-Fluorophenol Acros Organics Lot#A003610401; 3-Chlorophenol Acros Organics Lot#A013855901; 3-Bromo-5-fluorophenol Aldrich Lot#MKBF3319V; and 3-Chloro-5-fluorophenol Combi-Blocks Inc. Lot#L42454 and Lot#L54033, BePharm Limited Lot#0032554-16070101, Ark Pharm Lot#WG0032554-140708001, Oakwood Chemical Lot#013283113F.

Chromatographic conditions

A gas chromatography (GC) system was setup with the following:

Column: Rtx[®]-35 30-m × 0.25-mm, 1.0 μm film, S/N 1402372 cat. #10453 or Rtx[®]-65 30-m × 0.25-mm, 1.0 μm film, S/N 1394868 cat. #17053.

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Oven Program: 100°C to 210°C @10°C/min to 210°C (hold 14 min).

Inlet: Split Ratio 25:1, Temp. 240°C.

Detector: FID, Temp. 240°C.

Carrier Gas: Helium @ 1.0 mL/min.

Injection Volume: 1 µL.

Sample preparation

5.0 mg/mL Sample Solution: Transferred 50 mg of 3-chloro-5-fluorophenol to a 10-mL volumetric flask and diluted to volume with MeOH. Alternatively, acetonitrile (MeCN) may be used as a diluent.

Sensitivity Solution: Performed a 2000x dilution of the 5.0 mg/mL sample solution. For example, transferred 1.0 mL to a 100-mL volumetric flask then diluted to volume with MeOH and mixed thoroughly. Transferred 1.0 mL of the intermediate solution to a 20-mL volumetric flask then diluted to volume with MeOH and mixed.

Qualitative mixture solutions: Transferred 1 drop of each desired component to a 100-mL volumetric flask containing some MeOH, then diluted the volume with MeOH (For components in each mixture see Figure 3).

Equipment and software

The GC-FID system employed during these experiments was an Agilent Technologies 7890B GC System equipped with an Agilent 7693 Auto sample and an Agilent G4513A Injector. The GC columns discussed were purchased from the Restek® Corporation. The acquisition software utilized was Empower 3 licensed from the Waters Corporation.

Results and Discussion

Method Development: Due to the physical properties of 3-chloro-5-fluorophenol [6] GC-FID analysis was deemed the most appropriate analytical technique for development of a method which separates all of the regioisomer impurities as well as some previously known related impurities found within this raw material. One related compound mixture, two regioisomer mixtures, and a sample solution were prepared for GC column stationary screening (Figure 3). After a general GC column screen employing oven temperature gradient, it was found that all possible regioisomer impurities could be separated from each other and 3-chloro-5-fluorophenol utilizing an Rtx®-35 column (Figure 4).

However, the Rtx®-35 column was not able to cleanly separate the related compound mixture from regioisomer mixture #1 (see the region in boxed area of Figure 4). The Rtx®-35 stationary phase is composed of 35% diphenyl/ 65% dimethylpolysiloxane therefore to obtain better

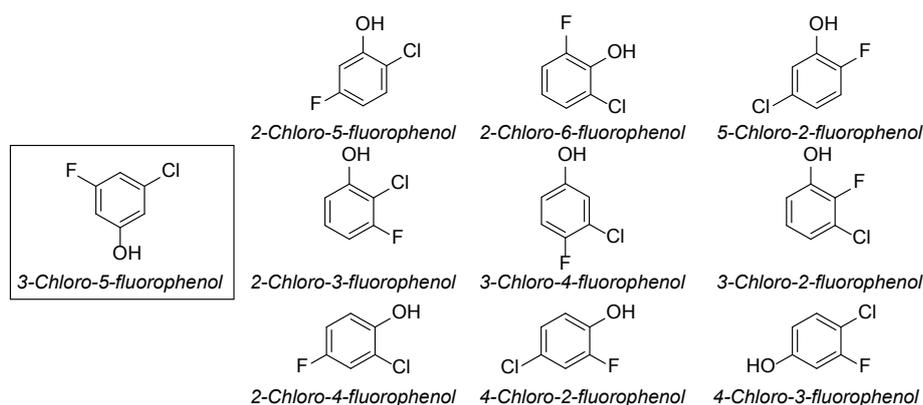


Figure 2: All Possible Regioisomer Impurities of 3-Chloro-5-fluorophenol.

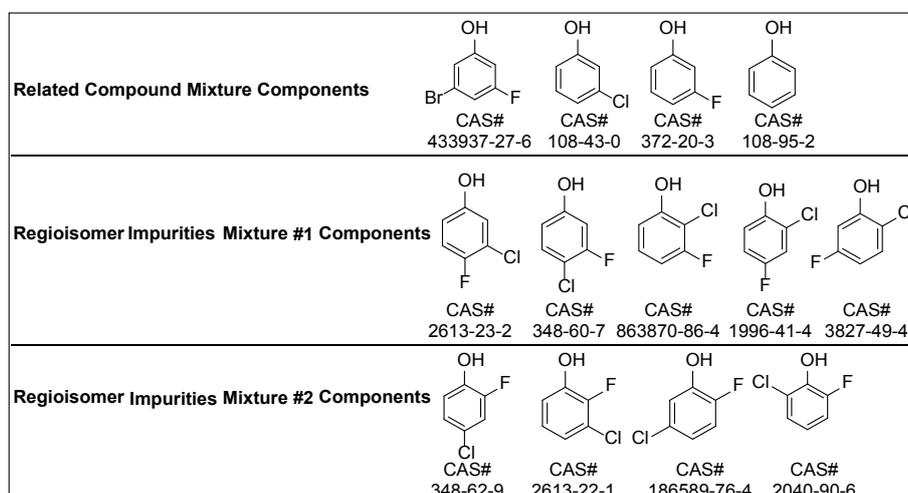


Figure 3: Structures of components in qualitative mixtures prepared for GC screening.

selectivity between the related compound mixture and regioisomer mixture #1 the stationary phase Rtx®-65 which contains 65% diphenyl to 35% dimethyl polysiloxane was attempted. Gratifyingly, this stationary phase with increased diphenyl content separated all related compounds as well as the nine possible regioisomer impurities (see the region in boxed area of Figures 5 and 6).

Method attributes: The method attributes (limit of detection, limit of quantitation, linearity, and carryover) for the impurity profile GC-FID method of 3-chloro-5-fluorophenol are shown in Table 1. Adequate detectability was achieved for the 0.001 mg/mL (0.02% with respect to (WRT) sample concentration) solution of 3-chloro-5-fluorophenol which gave greater than 3:1 signal to noise (S/N) for the 3-chloro-5-

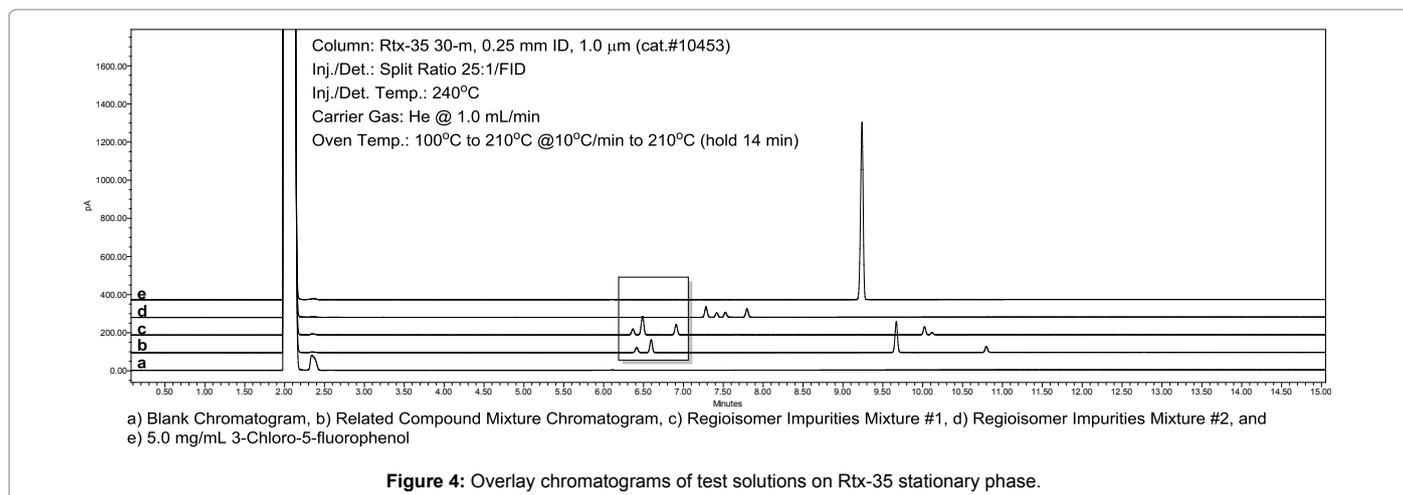


Figure 4: Overlay chromatograms of test solutions on Rtx-35 stationary phase.

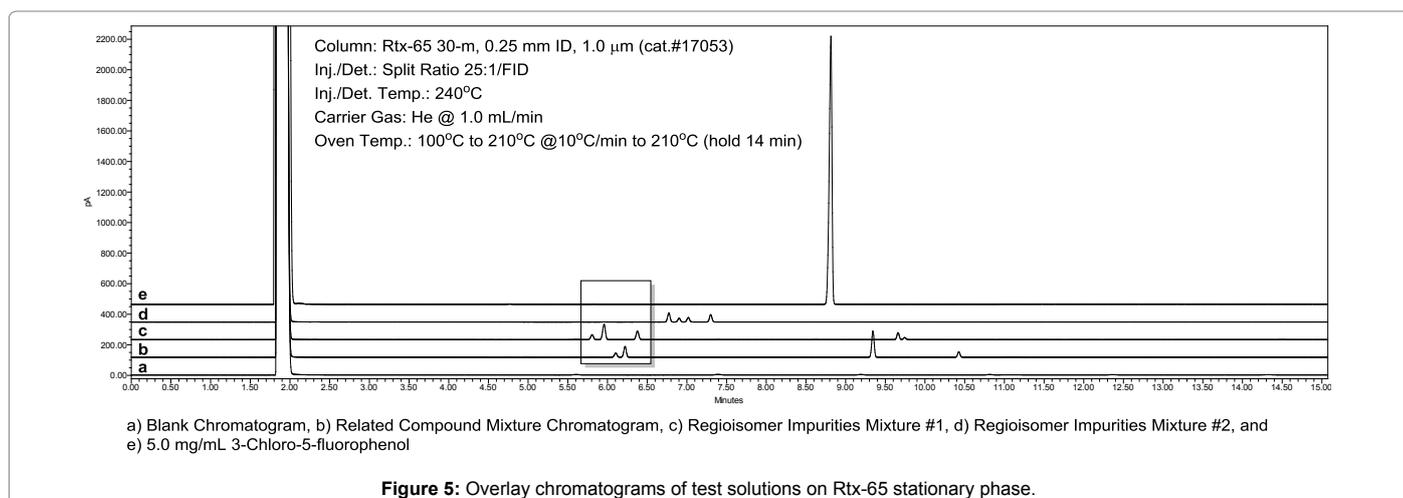


Figure 5: Overlay chromatograms of test solutions on Rtx-65 stationary phase.

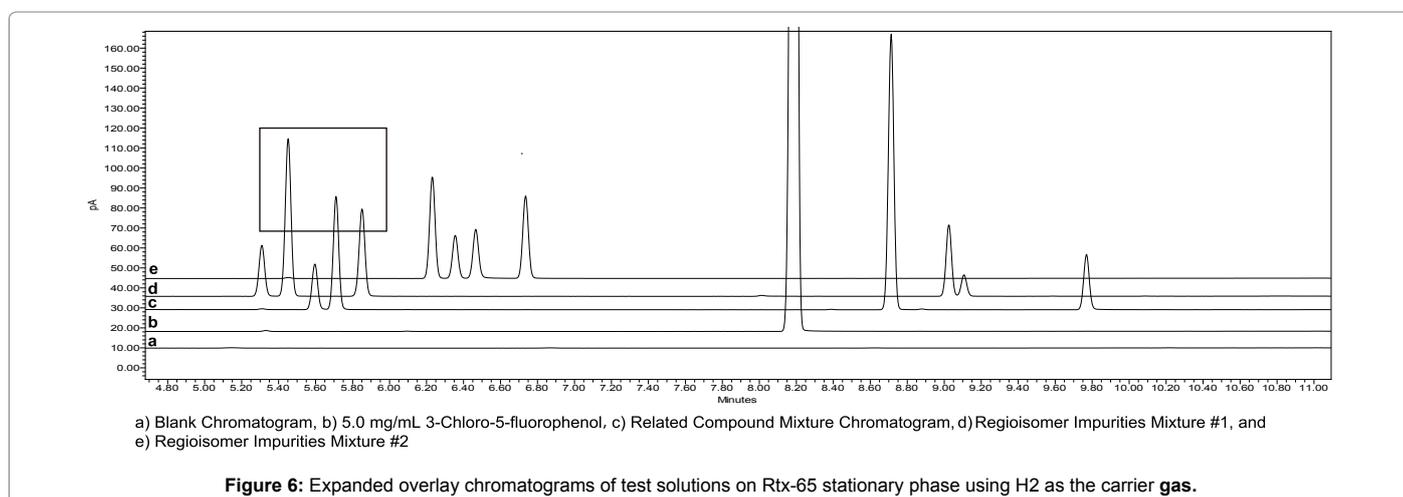


Figure 6: Expanded overlay chromatograms of test solutions on Rtx-65 stationary phase using H2 as the carrier gas.

fluorophenol (found 11 S/N) and is denoted as the reporting limit of detection for the method. The reporting limit of quantitation for this method was set to 0.05% sample solution of 3-chloro-5-fluorophenol since the S/N was greater than 15:1 ([7], found 29 S/N). Linearity assessment of 3-chloro-5-fluorophenol was conducted with six points over the concentration range of 0.001 mg/mL to 10.0 mg/mL which is equivalent to 0.02% to 200% of the target sample concentration of 5 mg/mL. Carryover of 3-chloro-5-fluorophenol was evaluated by performing a sample injection followed by a diluent injection and integration of the signal at the retention time of 3-chloro-5-fluorophenol. As shown in Table 1 all criteria set forth for limit of detection, limit of quantitation, linearity, and carryover method attributes were met.

Control Strategy: In order to develop a robust control strategy of the possible regioisomer impurities within the raw material 3-chloro-5-fluorophenol, samples were ordered from several commercially available manufacturers and their products were subjected to the developed GC-FID method. The area% results for these GC-FID analyses are in Table 2. Of the possible nine regioisomer impurities

Method Attribute	Criteria	Result
Limit of detection 0.0010 mg/mL (0.02%)	S/N (n=3) 3:1, % RSD none	11, 3.4%
Limit of quantitation 0.0025 mg/mL (0.05%)	S/N (n=3) ≥ 15:1, % RSD ≤ 15.0%	29, 0.4%
Linearity	Correlation Coefficient ≤ 0.99	1.000
Carryover	Less than the area of the LOD injection (0.02%)	0.007%

Table 1: Summary of the method attributes limit of detection, limit of quantitation, linearity, and carryover.

only three regioisomer impurities (2-chloro-5-fluorophenol, 2-chloro-6-fluorophenol, and 4-chloro-3-fluorophenol) were observed by retention time conformation from the four manufactures of 3-chloro-5-fluorophenol analysed. All area% values for the regioisomer impurities observed were below 0.10% area%. Therefore, we were confident that if we implemented an internal specification of no more than 0.15 area% for each of these regioisomer impurities within 3-chloro-5-fluorophenol the resulting impurities would not likely be of concern in downstream processing steps. Since regioisomers and their corresponding downstream intermediates may have different physical and chemical properties, their corresponding regioisomer intermediate levels may increase due to their inability to be rejected from the process but this scenario is typically a rare occurrence.

As for the other known impurities (phenol, 3-fluorophenol, 3-chlorophenol, and 3-bromo-5-fluorophenol) within the raw material 3-chloro-5-fluorophenol, all vendors contained at least one of these impurities. Since each vendor's manufacturing process of this raw material was not disclosed, we would recommend the identification of any impurity above 0.15 area% before use. Identification would allow the customer the ability to track the fate and purge of any unknown impurity above the 0.15 area% threshold and should be easily achieved by coupling the GC-FID method with MS technology [8-9]. In the case for unknown impurities RRT 0.24 and 0.26 in Table 2 at impurity levels above 0.15 area%, these impurities could be simply the processing solvents and/or starting materials for this manufacturer. We chose not to identify these peaks.

Robustness evaluation: To demonstrate the robustness of the analytical method the following method parameters were altered: carrier gas, diluent, and site location. Replacement of the carrier gas from

Manufacture			Combi-blocks, inc.	Combi-blocks, inc.	BePharm Limited	Oakwood Chemical	Ark Pharma
Lot/batch			L42454	L54033	0032554-16070101	13283	WG0032554-140708001
Name	CAS#	RRT	%Area	%Area	%Area	%Area	%Area
		0.24	0.75				
		0.26		0.39			
		0.30			0.03		0.03
		0.32			0.04		0.04
2-chloro-5-fluorophen	3827-49-4	0.66	0.05		0.03		0.02
Phenol	108-95-2	0.69				0.09	
3-fluorophenol	372-20-3	0.71				0.05	
		0.75	0.03				
2-Chloro-6-fluorophenol	2040-90-6	0.80				0.06	
		0.86				0.06	
		0.90			0.11		
		0.99			0.02		
3-Chloro-5-fluorophenol	202982-70-5	1.00	99.11	99.37	99.70	99.58	99.67
3-Chlorophenol	108-43-0	1.06				0.04	
		1.09				0.03	
4-Chloro-3-fluorophenol	348-60-7	1.11				0.04	
3-Bromo-5-fluorophenol	433937-27-6	1.19		0.22			
		1.21			0.04		0.04
		1.56				0.02	
		1.65			0.04		0.04
		1.72	0.06				
		1.92		0.02			
		2.45				0.02	

Table 2: Impurity Profile Data from Several Manufacturers of 3-Chloro-5 fluorophenol.

helium to hydrogen resulted in an overall decrease in retention time of all compounds with similar relative retention times (RRT) in respect to the retention time of 3-chloro-5-fluorophenol (Table 3). The RRT were determined by individual identification injections of each impurity onto the GC-FID system. Diluent evaluation was either performed by preparation of a sample or by a single blank injection. Acetonitrile as diluent appears acceptable since the same area% impurity profile was obtained for the 3-chloro-5-fluorophenol from Oakwood Chemical Lot 013283113F (Table 4). High boiling solvents like DMAc and NMP eluted in regions of interest and would interfere with the area% analysis if they were used as the diluent. To emphasize the robustness of the method, the method has been successfully utilized externally as well as internationally for the purpose of detection and quantitation of regioisomer impurities within the raw material 3-chloro-5-fluorophenol used in the synthesis of a potential API.

Proposed System Suitability Criteria

After evaluation of the collected data, the following minimal system suitability criteria are proposed to ensure proper detection and quantitation of the regioisomer impurities within 3-chloro-5-fluorophenol utilizing the described GC-FID method

- (1) The blank injection is free of significant interference at the retention times of 3-chloro-5-fluorophenol and known impurities.
- (2) The sensitivity solution (2000x dilution of the sample solution) should provide at least 10:1 S/N.

CAS#	He Carrier Gas		H ₂ Carrier Gas	
	RT (min)	RRT	RT (min)	RRT
3827-49-4	5.809	0.66	5.309	0.65
1996-41-4	5.959	0.68	5.452	0.67
108-95-2	6.103	0.69	5.596	0.68
372-20-3	6.222	0.71	5.711	0.70
863870-86-4	6.381	0.73	5.851	0.71
348-62-9	6.776	0.77	6.231	0.76
185689-76-4	6.902	0.79	6.355	0.78
2040-90-6	7.019	0.80	6.466	0.79
2613-22-1	7.302	0.83	6.736	0.82
202982-70-5(main)	8.784	1.00	80188	1.00
108-43-0	9.354	1.10	9.025	1.06
2613-23-2	9.664	1.10	9.025	1.10
348-60-7	9.744	1.11	9.107	1.11
433937-27-6	10.426	1.19	9.769	1.19

Table 3: Retention Time and Relative Retention Time Comparison Between the Carrier Gasses Helium and Hydrogen.

Oakwood Chemical Lot 013283113F		MeOH	MeCN
Name	RRT	%Area	%Area
108-95-2	0.69	0.09	0.09
372-20-3	0.71	0.05	0.05
2040-90-6	0.80	0.06	0.06
	0.86	0.06	0.06
202982-70-5	1.00	99.58	99.57
108-43-0	1.06	0.04	0.04
	1.09	0.03	0.03
348-60-7	1.11	0.04	0.05
	1.56	0.02	0.02
	2.45	0.02	0.02

Table 4: Area% Comparison Between the Diluents MeOH and MeCN.

Conclusion

A general GC-FID area% method employing an Rtx®-65 column has been developed for the detection and quantitation of all possible regioisomer impurities of 3-chloro-5-fluorophenol. The method as described has been shown to have a reporting quantitation limit of 0.05% and a reporting detection limit of 0.02% WRT a sample concentration of 5.0 mg/mL.

Notes and References

1. The International Council for Harmonisation (ICH) (2006) Q3A (R2): Impurities in New Drug Substances (ICH, October 2006).
2. The United States Pharmacopeia and The National Formulary (USP-NF), General Chapter: <1086> Impurities in Drug Substances and Drug Products.
3. European Pharmacopoeia (EP), General Texts: 5.10. Control of Impurities in Substances for Pharmaceutical Use and references within text (04/2012:51000).
4. Regioisomer impurities are impurities that have the same carbon skeleton connectivity but differ in the connectivity of the functional groups onto the carbon skeleton.
5. ICH, Q11: Development and Manufacture of Drug Substances (ICH, may 2011)
6. SciFinder® A CAS Solution search of 3-Chloro-5-fluorophenol CAS# 202982-70-5, MW 146.55 g/mole, B Pt (predicted) 206 ± 20°C at 760 Torr, physical state liquid at room temperature.
7. A goal to have the S/N for the reporting limit of quantitation solution of at least 15:1 can aid in transferring the method to other laboratories.
8. Ecker J, Scherer M, Schmitz G, Liebisch G (2012) A rapid GC-MS method for quantification of positional and geometric isomers of fatty acid methyl esters. Journal of Chromatography B 897: 98-104.
9. Fariña L, Boido E, Carrau F, Dellacassa E (2007) Determination of volatile phenols in red wines by dispersive liquid-liquid microextraction and gas chromatography-mass spectrometry detection. Journal of Chromatography A 1157: 46-50.