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Development and validation of a specific and sensitive GC-FID method for the determination of impurities in 5-chlorovaleroyl chloride

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ABSTRACT

5-Chlorovaleroyl chloride (5-CVC) is commonly used as an alkylating agent in the synthesis of pharmaceutical intermediates, active ingredients, as well as other specialty chemicals. It is critical to monitor the impurities present in 5-CVC as they may have a direct impact on the impurity profile and quality of the final product. This paper describes the development and validation of a GC-FID method for the analysis of low level impurities of 5-CVC. This is the first method reported in the literature for the impurity determination of 5-CVC. The results of GC method development, with and without sample derivatization, are presented. The final method uses methanol for derivatization and separates methyl esters of 5-CVC and the key impurities, 4-pentenoyl chloride, 4-chlorovaleroyl chloride, 5-chlorohexanoyl chloride, and 4-methyl-5-chlorovaleroyl chloride. 3-Methoxypyridine was used in the sample solvent to enable the detection of 5-chlorovaleric acid (5-CVA) which is the major degradant of 5-CVC. The method was validated for specificity, linearity, accuracy, precision, sensitivity, and robustness. This simple and robust GC approach may be applicable to impurity analysis of other acid chlorides or acid halides.

1. Introduction

5-Chlorovaleroyl chloride (5-CVC) is commonly used as an alkylating agent in the synthesis of pharmaceutical intermediates, active ingredients, and other specialty chemicals. The impurities in 5-CVC vary depending upon the manufacturer and the chemical process used to produce 5-CVC. If these impurities also contain the acid chloride moiety, they may also alkylate pharmaceutical intermediates in the synthetic sequence and thus be the origin of impurities in the active pharmaceutical ingredient (API). Therefore, in order to control the quality of an API, a specific, sensitive, and accurate method is needed for the quantitation of low level impurities in 5-CVC.

In general, direct chromatographic quantitation of acyl halides is problematic due to their high reactivity in the presence of water. The hydrolysis of acyl halides by water to the corresponding carboxylic acids is facile and fast [1]. The quantitation of acyl halides is primarily achieved by gas or liquid chromatography following acylation of an alcohol or amine. During 1960s, there were a few reports of gas chromatographic separation and quantitation of acyl chlorides using the acylation approaches [2–4]. Later, more derivatization procedures were developed for HPLC separation and quantitation of acyl chlorides [5,6] and similar approaches have been extended to thioureas [7,8].

Most of these derivatization procedures used amines and alcohols with an acidic aqueous extraction to remove excess reagent. As a result, the hydrolysis product of acyl chloride, the carboxylic acid, was separated from the amide or ester derivative and not routinely measured. An additional method was generally developed to quantitate the corresponding carboxylic acid level present in an acyl chloride. An exception is the approach of Niedermayer [3], who did not extract the excess alcohol. It was observed that the carboxylic acid decreased in concentration with time because of acid-catalyzed esterification following derivatization, and hence the samples required immediate analysis after preparation.

The corresponding carboxylic acid of an acyl chloride is not only the hydrolysis product (degradant), but is often used as a starting material in the synthesis of an acyl chloride. Therefore, a specific and sensitive method to fully resolve and quantitate potential low level impurities in an acyl chloride, in particular, the corresponding carboxylic acid, was needed for quality control purposes.

During the process development of an API at Bristol-Myers Squibb which used 5-CVC in the chemical synthesis, 15 batches of 5-CVC from 11 different manufacturers were evaluated for quality. A total of eight impurities with levels of greater than 0.05% area percent were identified by GC/MS. Among the eight impurities, one was the hydrolysis product of 5-CVC, 5-chlorvaleric acid (5-CVA), and four others contained the highly reactive acid chloride moiety

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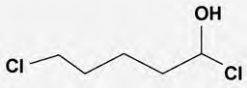
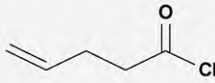
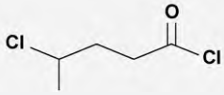
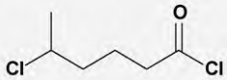
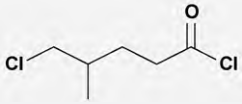
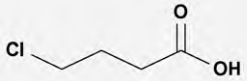
Table 1
Chemical names, structures, and abbreviations of 5-CVC and the major impurities/degradant.

Chemical name	Structure	Abbreviation
5-Chlorovaleroyl chloride		5-CVC
4-Pentenoyl chloride		CVC-ene
4-Chlorovaleroyl chloride		4-CVC
5-Chlorohexanoyl chloride		5-Me CVC
4-Methyl-5-chlorovaleroyl chloride		4-Me CVC
5-Chlorovaleric acid		5-CVA

and were present in most of the batches manufactured by different vendors. The other three impurities were identified as 3-picoline, γ-valerolactone, and δ-valerolactone with no acid chloride moiety, and were only detected in two batches manufactured by one vendor. It was further determined that the acid chloride impurities in 5-CVC were carried through the synthesis and converted to the corresponding impurities in final isolated API. Therefore, our method development focused on separation and quantitation of these four key and typically found acid chloride impurities as well as the hydrolysis product of 5-CVC, 5-chlorovaleric acid (5-CVA). The chemical names, abbreviations, and structures are listed in Table 1.

The method reported here may find utility in both the pharmaceutical and chemical industries, and it may potentially be applied to the purity/impurity analysis of other acid chlorides or acid halides.

2. Materials and methods

2.1. Materials

Batches of 5-CVC were purchased from different manufacturers including Sigma–Aldrich (St. Louis, MO, USA), BASF (Evionnaz, Switzerland), Isochem (Vert-le-petit, France), and Varsal (West Chester, PA, USA). CVC-ene, 5-CVA, and 3-methoxypyridine were purchased from Sigma–Aldrich. 4-CVC, 5-Me CVC, and 4-Me CVC were produced by Bristol-Myers Squibb (New Brunswick, NJ, USA). Chromatographic grade dichloromethane (DCM) and HPLC grade methanol were purchased from Burdick & Jackson (Muskegon, MI, USA).

2.2. Sample preparation

All the samples used for method development and validation were prepared volumetrically. The target sample concentration of 5-CVC was 4.0%. The 5-CVC sample solution was prepared using the following procedure: using a 1-mL gastight syringe or Class A 1-mL glass pipette, 1 mL of 5-CVC was added slowly (drop by drop) into a 25-mL volumetric flask containing 5 mL of methanol and 1 mL of 3-methoxypyridine with occasional swirling. Slow addition of 5-CVC prevents excessive generation of heat and fumes. This procedure was carried out in a fume hood, and the mixture was allowed to sit in the hood for 15–20 min to cool and ensure completion of the derivatization reaction. The reaction mixture was then diluted to volume with DCM and mixed well before injection. A blank solution was prepared by adding 5 mL methanol and 1 mL 3-methoxypyridine into a 25-mL volumetric flask and diluting to volume with DCM. A solution containing 4% (v/v) 5-CVC spiked with the authentic materials of CVC-ene, 4-CVC, 4-Me CVC, 5-Me CVC and 5-CVA at levels of 0.4–0.8% (v/v) was prepared in a similar fashion as a sample solution and was used for system suitability testing.

2.3. Equipment

All analyses were performed on an Agilent 6890 capillary GC system (Wilmington, DE, USA) equipped with a split injector and a flame ionization detector. An Rtx-1701 Crossbonded column (14% cyanopropylphenyl-86% methyl polysiloxane), 30 m × 0.25 mm i.d., 1 μm film thickness from Restek (State College, PA, USA) was used.

2.4. Chromatographic conditions

All separations were performed using the Rtx-1701 Crossbonded column with a temperature gradient, constant flow rate, and split injection. The oven temperature program was set as follows: 80 °C for 0 min, increase at 20 °C/min to 120 °C, hold at 120 °C for 5 min, increase at 40 °C/min to 140 °C, and hold at 140 °C for 2 min, increase at 40 °C/min to 250 °C, and hold at 250 °C for 5.75 min. Run time was approximately 18 min. Injector and detector temperatures were set at 250 °C. Helium was used as the carrier gas at a flow rate of 2.2 mL/min. Sample was injected by the instrument's autosampler with injection volume of 1.0 μL and dichloromethane as the syringe cleaning solvent between injections. The split ratio was set at 50:1 (split flow ~120 mL/min) and a 4-mm i.d., deactivated open-glass tube liner packed with fused silica wool was employed.

2.5. Suggested system suitability criteria

For this method, the following system suitability criteria were established: RSD of the peak area of 5-CVC ≤ 10.0% from at least six injections of the system suitability solution. Resolution between 5-Me CVC and 4-Me CVC peaks ≥ 4.0.

2.6. Precautions

Since the analytes and reagents used for sample preparation are corrosive and volatile, prepare samples in a fume hood and use appropriate personal protective equipment such as gloves and safety glasses. Precautions during the derivatization reaction are described in Section 2.2.

3. Results and discussions

3.1. Method development

3.1.1. Analysis of 5-CVC and impurities without derivatization

Although not an approach commonly used, a direct injection of 5-CVC without sample treatment was initially evaluated. A chromatogram overlay of a blank, the first and the fifth injections of a 5-CVC sample is shown in Fig. 1. The baseline rose significantly after

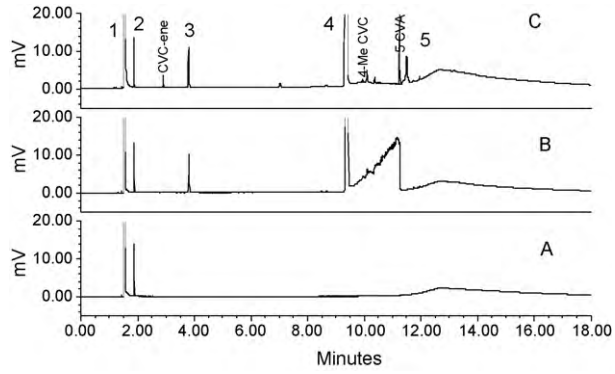


Fig. 1. Chromatogram of direct injection of 5-chlorovaleroyl chloride (5-CVC). A: blank (dichloromethane); B: 1st injection of sample; C: 5th injection of sample. Peaks 1 and 2: dichloromethane and related impurity; peak 3: 3-picoline impurity in sample; peak 4: 5-CVC (4.0% v/v, corresponding to ~0.96 μg injected on the column); peak 5: unknown impurity in sample.

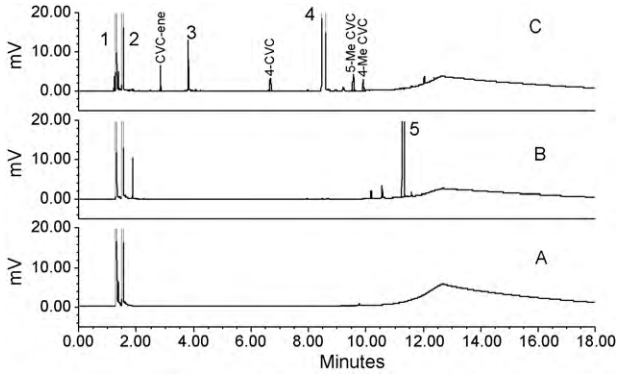


Fig. 2. Chromatogram of 5-chlorovaleroyl chloride (5-CVC) and its impurities as methyl ester derivatives. A: blank (methanol and dichloromethane); B: 5-CVA (1.0%) in methanol and dichloromethane; C: sample in methanol and dichloromethane. Peak 1: methanol; peak 2: dichloromethane; peak 3: 3-picoline impurity in sample; peak 4: 5-CVC (4.0% v/v, corresponding to ~0.96 μg injected on the column); peak 5: 5-CVA.

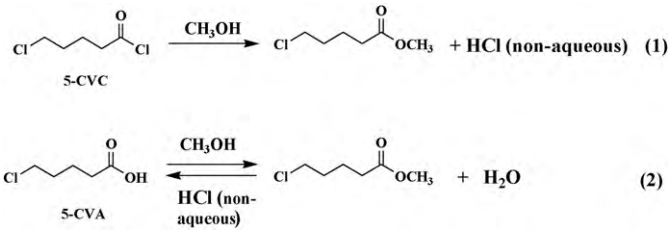
the 5-CVC peak elution at ~9.5 min for the first injection (Fig. 1B). This baseline shift was hypothesized to be due to reaction of 5-CVC with the stationary phase and/or potential degradation of the stationary phase materials. Although the baseline improved for the subsequent injections (as shown in Fig. 1C), inconsistent peak areas for 5-CVC and for impurities were obtained for the repeated injections. The peak area for the low level impurities CVC-ene, 4-CVC, 5-Me CVC and 4-Me CVC increased as the sample was repeatedly injected. The peaks were not detected for the initial three injections but were detected after the third injection, and the peak area increased as the number of injections increased. This trend indicates that interaction/degradation of these low level acid chloride impurities occurred in the injector liner or on-column for the initial injections. After "conditioning" the system by repeated injections of the sample, the impurity response increased for the subsequent injections. These observations led to the exploration of a procedure using derivatization to give less reactive analytes.

3.1.2. Analysis of 5-CVC and impurities as methyl ester derivatives

Reaction of 5-CVC and related compounds with methanol was performed. The sample was prepared the same way as described in Section 2.2 except that 5-CVC was added into pure methanol (no 3-methoxypyridine) to generate the methyl ester derivative of 5-CVC and its related impurities. The peaks were identified by GC/MS. As shown in Fig. 2C, the baseline as well as the peak shape of these methyl ester derivatives improved significantly compared with the direct injection without derivatization. More importantly, good recovery and precision were obtained for the impurities. The peak areas for the low level impurities in the sample were consistent for repeated injections indicating no on-column interaction/degradation of these impurities. However, 5-CVA, the major hydrolysis degradant of 5-CVC, was not observed with this derivatization procedure. This was confirmed by spiking with different levels of 5-CVA in 5-CVC. The 5-CVA peak was not detectable in all the spiked samples including a sample spiked with 5% 5-CVA relative to 5-CVC. For comparison, a sample of 1% 5-CVA diluted in methanol and DCM was injected using the same GC conditions. As shown in Fig. 2B, the peak eluted at 11.3 min was confirmed by GC–MS as 5-CVA. However, a 5-CVC sample spiked with 1% 5-CVA showed no detectable 5-CVA peak.

The hypothesis for the failure of recovery for 5-CVA in 5-CVC using methanol as the derivatization reagent was that 5-CVA was converted to its methyl ester derivative, which is the same product as the methyl ester derivative of 5-CVC. While organic acids are not readily converted to their respective methyl ester in the presence of an alcohol, they will convert under non-aqueous acidic conditions known as the Fischer Esterification. The equilibrium of the Fischer Esterification reaction may be influenced by either removing water from the reaction mixture or employing an excess of reactants [9].

In the sample preparation used here, non-aqueous hydrochloric acid (HCl) was generated from the derivatization of 5-CVC with methanol as shown in Scheme (1). The in situ generated HCl facilitated the derivatization of 5-CVA with methanol to form the methyl ester which is the same product as the methyl ester derivative of 5-CVC as shown in Scheme (2).



Since 5-CVA is a major hydrolysis product of 5-CVC, the analysis of 5-CVA should be included in the method for the purpose of quality control. Thus, an alternative approach was required to analyze 5-CVA in the presence of 5-CVC.

3.1.3. Analysis of 5-CVC and impurities as methyl ester derivatives with addition of 3-methoxypyridine

To test the hypothesis of free carboxylic acid being converted to the methyl ester derivative due to excess methanol in the presence of in situ liberated non-aqueous HCl, a base (pyridine) was added to methanol in the flask prior to the addition of 5-CVC to neutralize the liberated HCl. A similar approach was tried by Machado et al. [6] for the derivatization reaction of hydrocinnamoyl chloride with ethanol. However, in that work the corresponding pyridinium chloride salt was insoluble in the ethanol solution. The pyridinium chloride salt generated in our procedures was soluble in methanol/dichloromethane solution. Injection of a sample spiked with 5-CVA prepared using this procedure showed an observable 5-CVA peak. However, pyridine co-eluted with a key derivatized impurity (CVC-ene). Other bases including triethylamine (TEA), diethylamine (DEA), 4-ethylpyridine, 3-butylpyridine, and 3-methoxypyridine were further evaluated regarding the recovery of 5-CVA and the separation of the impurities from the base additives. Samples prepared with TEA and DEA showed a very poor baseline (data not shown) which inhibited the accurate integration

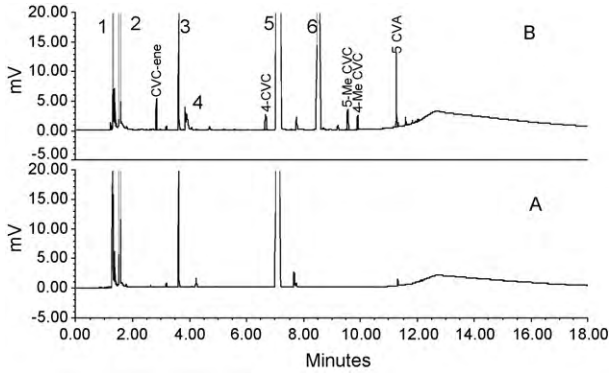


Fig. 3. Chromatogram of 5-chlorovaleroyl chloride (5-CVC) and its impurities as methyl ester derivatives with addition of 3-methoxypyridine in the sample. A: blank (methanol + dichloromethane + 3-methoxypyridine), B: sample. Peak 1: methanol; peak 2: dichloromethane; peak 3: unknown impurity in 3-methoxypyridine; peak 4: 3-picoline impurity in sample; peak 5: 3-methoxypyridine; peak 6: 5-CVC (4.0% v/v, corresponding to ~0.96 μg injected on the column).

of impurity peaks. Among the various pyridine derivatives studied, 3-methoxypyridine was chosen as the base to neutralize the liberated HCl. All the peaks from 3-methoxypyridine and its observed impurities were well separated from the five impurities of 5-CVC (CVC-ene, 4-CVC, 5-Me CVC, 4-Me CVC, and 5-CVA), as shown in Fig. 3B. The amount of 3-methoxypyridine added was optimized to achieve quantitative recovery for the impurities. Experiments with different volumes of 3-methoxypyridine showed that HCl must be completely neutralized with excess methoxypyridine to achieve quantitative recovery of 5-CVA. The final method used 1 mL of 3-methoxypyridine for 1 mL of 5-CVC in 25 mL solution which is equivalent to ~125% (molar ratio) of the base compared to the amount of HCl generated following the derivatization reaction. For peak identification, a solution containing 4% 5-CVC spiked with authentic samples of CVC-ene, 4-CVC, 4-Me CVC, 5-Me CVC, and 5-CVA at levels of 0.4–0.8% relative to 5-CVC was injected, and the peak identities were confirmed by GC/MS.

3.2. Method validation

In order for the above-developed method to be used in Quality Control labs as a routine analysis procedure, method validation was performed with criteria according to ICH guideline, Q2 (R1) [10]. The method was validated for specificity, linearity of the methyl ester derivatives of 5-CVC and its impurities (CVC-ene, 4-CVC, 5-Me CVC, 4-Me CVC, and 5-CVA), accuracy (spike and recovery), sensitivity, repeatability of replicate injections and replicate sample preparations, intermediate precision, stability of the sample solution, and robustness of the method to withstand deliberate changes in the chromatographic conditions.

3.2.1. Specificity

The specificity of the GC method is illustrated in Fig. 3B where the methyl ester derivatives of 5-CVC and the key impurities (CVC-ene, 4-CVC, 5-Me CVC, 4-Me CVC) and 5-CVA were completely separated from each other, and from the sample matrix peaks (methanol, dichloromethane, 3-methoxypyridine and its impurities). Injection of a blank solution after sample injections did not produce a detectable peak at the retention time of 5-CVC indicating no observable carry-over. The resolution for the critical pair impurities, 5-Me CVC and 4-Me CV, was 5.7.

3.2.2. Linearity

Linearity of the 5-CVC methyl ester response was determined from 1.8 to 6.0% (v/v). This range corresponds to approximately 45–150% of the nominal sample concentration of 4.0%. Linearity of the response for the impurities (CVC-ene, 4-CVC, 5-Me CVC, 4-Me CVC, and 5-CVA) was conducted in the range of 0.0016–0.16% (v/v). Analysis of the data using a Least Squares Regression indicated that the data fit a linear model with $r^2 > 0.999$ and with y-intercepts not significantly different from zero ($p < 0.05$). To determine the correlation of area % from the GC chromatogram to actual v/v % (volume impurity/volume 5-CVC), the relative response factors (RRF) of the impurities to 5-CVC were calculated as ratios of the impurity response to that of 5-CVC. The RRF values were determined to be 1.05 for CVC-ene, 0.94 for 4-CVC, 1.08 for 5-Me CVC, 1.02 for 4-Me CVC, and 0.854 for 5-CVA. Given the RRFs were within ±15% of 5-CVC (an acceptably small deviation from unity), RRFs of 1.0 were assigned to the impurities for the routine use of this method, and the area % determined from the GC chromatogram is taken to be equivalent to the v/v % (volume of impurity/volume of 5-CVC) without further correction.

3.2.3. Accuracy-impurity spike and recovery

The accuracy of the method for the methyl ester derivatives of CVC-ene, 4-CVC, 5-Me CVC, 4-Me CVC, and 5-CVA in 5-CVC was determined with spiked sample recovery experiments. The above impurities were spiked into 5-CVC in triplicate at three different levels. The concentration of 5-CVC for all the spiking experiments was at a working concentration of 4.0% in diluent. The accuracy results are shown in Table 2. The results show adequate individual recoveries in the range of 87.9–122.8% with average recoveries

Table 2
Recovery of 5-CVC impurities from spiked samples.

Spiking level	Impurity (methyl ester derivative)	Conc. (% relative to 5-CVC)	Recovery % Prep 1	Recovery % Prep 2	Recovery % Prep 3	Average recovery (%)/%RSD
Low	CVC-ene	0.06	104.1	119.8	97.0	107.0/10.9
	4-CVC	0.06	111.2	120.9	87.9	106.7/15.9
	5-Me CVC	0.08	103.9	105.3	107.0	105.4/1.5
	4-Me CVC	0.04	111.4	112.3	110.4	111.4/0.9
	5-CVA	0.8	111.8	120.8	122.8	118.5/4.9
Medium	CVC-ene	0.15	97.2	105.0	105.3	102.5/4.5
	4-CVC	0.15	107.4	101.9	100.4	103.2/3.6
	5-Me CVC	0.20	109.7	108.7	106.5	108.3/1.5
	4-Me CVC	0.10	113.2	111.6	110.2	111.7/1.3
	5-CVA	2.0	114.7	113.2	113.0	113.6/0.8
High	CVC-ene	0.18	103.4	96.5	92.6	97.5/5.6
	4-CVC	0.18	114.5	101.7	96.1	104.1/9.1
	5-Me CVC	0.24	110.3	106.5	109.0	108.6/1.8
	4-Me CVC	0.12	114.7	110.0	109.8	111.5/2.5
	5-CVA	2.4	112.9	113.3	105.9	110.7/3.7

Table 3
Method precision: impurities determined (area % relative to 5-CVC) in sample preparations by different analysts on different days and analyzed on different instruments.

Day/analyst/Instrument	Prep	CVC-ene	5-CVC	5-Me CVC	4-Me CVC	5-CVA
	1	0.27	0.31	0.38	0.19	0.28
1/1/1	2	0.27	0.31	0.38	0.19	0.28
	3	0.27	0.31	0.38	0.19	0.29
	1	0.27	0.31	0.38	0.19	0.29
2/1/1	2	0.27	0.31	0.38	0.19	0.31
	3	0.27	0.31	0.38	0.19	0.33
	1	0.27	0.31	0.38	0.19	0.35
3/2/2	2	0.27	0.31	0.38	0.19	0.34
	3	0.27	0.31	0.38	0.19	0.39
Grand mean		0.27	0.31	0.38	0.19	0.32
%RSD between day		0.0	0.0	0.0	0.0	11.7
%RSD within day		0.0	0.0	0.0	0.0	6.1

ranging from 97.5 to 118.5%. The relatively higher values for recovery of 5-CVA may be due to moisture-induced hydrolysis of 5-CVC during sample preparation. For the same reason, relatively higher levels of 5-CVA (0.8–2.4%) were spiked in 5-CVC for the accuracy study.

3.2.4. Precision

The precision for repeat injections and sample preparations was validated for all key impurities (CVC-ene, 4-CVC, 5-Me CVC, 4-Me CVC, and 5-CVA). The %RSDs of peak responses of the methyl esters of 5-CVC and the impurities for six injections were determined to be in the range of 0.6–1.9% (data not shown). The %RSDs of retention times of the methyl esters of 5-CVC and the impurities for six injections were in the range of 0.1–0.4%. The method precision was also assessed by analyzing three batches of 5-CVC on three different days by two different analysts using two different instruments. The results (area % of impurity relative to 5-CVC) shown in Table 3 for one batch of 5-CVC, verify the repeatability of the method.

3.2.5. Sensitivity

The detection limit (DL) and quantification limit (QL) for the key impurities were estimated based on the standard deviation of the response (σ) and the slope (S) from the impurity linearity calibration curves. The standard deviations of these impurities were determined from six injections of the lowest level from the impurity linearity study. The DL and QL as v/v % (volume of impurities/volume of solution) were calculated according to the equations below. Using RRFs of 1.0 and 4.0% (v/v) as the concentration of 5-CVC, the DL and QL were then calculated as area % relative to 5-CVC, and are listed in Table 4. These results demonstrate that the sensitivity of the method is more than adequate for the determination of impurities with a reporting limit of 0.03% relative to 5-CVC (a typical level to which these impurities may need to be controlled in a pharmaceutical process).

$$\mathrm{DL} = \frac{3.3\sigma}{S} \qquad \mathrm{QL} = \frac{10\sigma}{S}$$

Table 4
Estimated DL and QL for 5-CVC impurities.

	CVC-ene	4-CVC	5-Me CVC	4-Me CVC	5-CVA
DL (area %)[a]	0.0055	0.0018	0.0065	0.0020	0.0023
QL (area %)[a]	0.0175	0.0050	0.0200	0.0050	0.0075

[a] Area % of impurities relative to 5-CVC.

3.2.6. Solution stability

The stability of the derivatized sample solution of 5-CVC was assessed by analyzing a sample solution on the day of preparation, and after storage for 2 and 5 days at room temperature/room light, and 5 °C protected from light. The results, as shown in Table 5, indicate the sample solution is stable for at least 5 days stored at both conditions of room temperature/room light, and 5 °C/protected from light. The solution stability extended beyond 5 days was not tested.

Table 5
Solution stability of 5-CVC and its impurities.

	Initial (area %)	Day 2		Day 5	
		Room temp/room light (area %)	5 °C/protected from light (area %)	Room temp/room light (area %)	5 °C/protected from light (area %)
CVC-ene	0.27	0.27	0.27	0.27	0.27
4-CVC	0.31	0.31	0.31	0.31	0.31
5-CVC	97.79	97.79	97.79	97.79	97.79
5-Me-CVC	0.38	0.38	0.38	0.38	0.38
4-Me-CVC	0.20	0.19	0.20	0.20	0.19
5-CVA	0.30	0.27	0.28	0.28	0.28

Table 6
Summary of method robustness testing results.

Parameter	Method condition	Method variation	Resolution (5-Me CVC and 4-Me CVC)	Tailing factor (5-CVC)	Plate count (5-CVC)
Injector temperature (°C)	250	±5	5.79/5.75/5.75	0.59/0.60/0.59	82297/95574/80637
Initial oven temperature (°C)	80	±2	5.72/5.75/5.77	0.59/0.60/0.59	84690/95574/79035
Flow rate (mL/min)	2.2	±0.1	5.76/5.75/5.72	0.59/0.60/0.60	80136/95574/86071
Split flow (mL/min)	110	±4	5.80/5.75/5.74	0.59/0.60/0.60	76726/95574/86707
Column	N/A	3 lots	5.75/5.86/5.52	0.60/0.59/0.60	82034/95574/78621

3.2.7. Method robustness—chromatographic conditions

The reliability of the analysis with respect to variation in the chromatographic conditions was tested by making deliberate changes to the method as described in Table 6 and monitoring the effect on resolution of the critical pair of peaks (5-Me CVC and 4-Me CVC), tailing factor, and plate count of the main peak (5-CVC) using the system suitability solution. The results are summarized in Table 6 indicating that there were no significant changes of the chromatographic figures of merit with the deliberate chromatographic parameter changes in the ranges stated. Based on these results, the chromatographic parameters are considered to be robust.

4. Conclusions

This is the first method reported in the literature for the separation and quantitation of 5-CVC and its impurities/degradants following a derivatization with methanol and 3-methoxypyridine. The GC-FID method is specific, linear, sensitive, accurate, precise, and robust. This approach may potentially be applied to the purity/impurity analysis of other acid chlorides or acid halides.

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