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Development of a Triphenylmethyl Alkylation Pre-Column Derivatization Method for HPLC Quantitative Analysis of Chemically Unstable Halogenated Compounds

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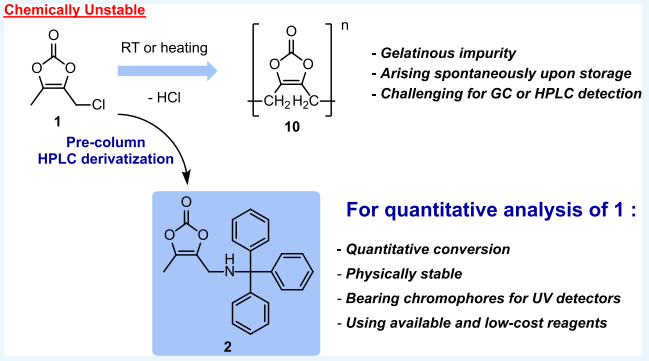
ABSTRACT: The primary limitations of the quantitative analysis of thermally labile halogenated compounds by traditional gas chromatography (GC) are the inadequacy of identifying the insufficiently volatile impurity (often with a high boiling point) and the difficulty in obtaining a standard substance with a reliable standardized assay. Taking the 4-(Chloromethyl)-5-methyl-1,3-dioxol-2-one (DMDO-Cl, **1**) as an example, we reported a triphenylmethanamino-derivatization method to overcome the challenges of the assay determination of such species. During the quantification of **1**, the presence of GC-undetectable polymeric impurity **10** poses a critical challenge in assessing the material quality. Moreover, the standard substance of **1** is not available on the market due to its inherent instability during storage and handling, further complicating the quantitative analysis. In this work, a precolumn HPLC-UV derivatization method based on triphenylmethanamino-alkylation was developed to quantitatively analyze **1**. The resulting derivative **2** exhibits excellent crystallinity and superior physical and chemical stability and possesses effective chromophores for UV detection. The conversion from analyte **1** to derivative **2** demonstrates desirable reactivity and purity, facilitating quantitative analysis using the external standard method. The chemical derivatization-chromatographic detection method was optimized and validated, demonstrating its high specificity, good linearity, precision, accuracy, and stability. This method offers a valuable alternative to the general quantitative NMR (qNMR) detection technique, which exhibits reduced specificity in the presence of increased levels of impurities in compound **1**.



KEYWORDS: *chemical derivatization, pre-column derivatization, HPLC, quantitative analysis, triphenylmethanamine, halogenated compounds*

INTRODUCTION

It is well-known that the stability of analytes is an important preanalytical variable for quantitation in chromatographic analysis [gas chromatography (GC) or high-performance liquid chromatography (HPLC)]. The quantitative determination of thermally unstable or highly reactive compounds often faces the typical following challenges: (i) spontaneous degradation or partial thermal decomposition happens during the analysis process, leading to inaccurate results; (ii) stable reference standards for assay calculation are often difficult to obtain; (iii) there is a lack of universal solutions (unique solution for each specific case). There are numerous factors contributing to the instability of the analyte during the analytical process;[1] however, there is a scarcity of widely applicable methods for cases characterized by the intrinsic instability of the analyte itself.

Quantitative nuclear magnetic resonance (qNMR) is a universal method for the determination of various compounds. In most cases, it avoids compound degradation during the quantitative NMR detection process, but it is limited to relatively low sensitivity, the lack of selectivity/specificity caused by the inherent resonance overlap, and the requirement of manual integration.[2] As a stopgap measure, the derivatization of easily degradable compounds into stable substances for chromatographic quantitative detection is a preferred solution, enabling the analyst to analyze a wide variety of compounds by GC, GC−MS, HPLC, and LC−MS that are unstable for these techniques. If the compound is ideally reactive, derivatization is relatively straightforward, such as acylation and silylation commonly used for −OH, −SH, and −NH groups.[3] Though various derivatization methods of halogenated compounds have been well developed,[4,5] the analysis of chemically unstable halogenated compounds is still challenging, as (i) they are

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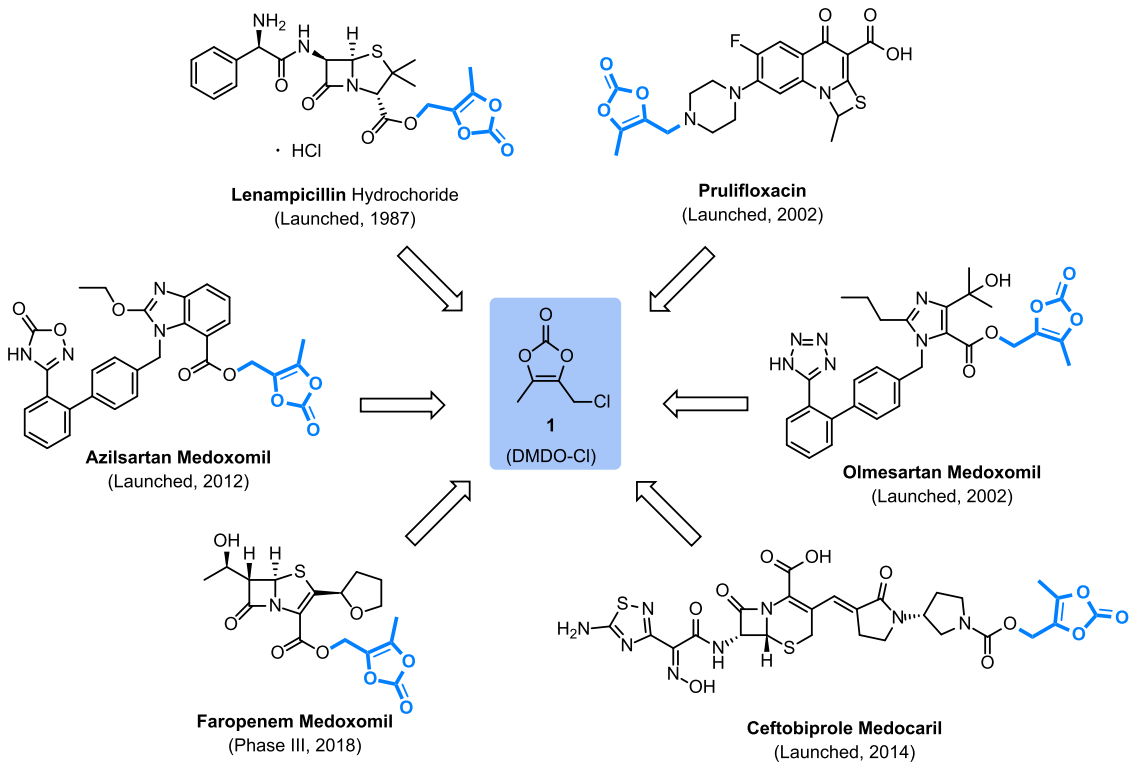
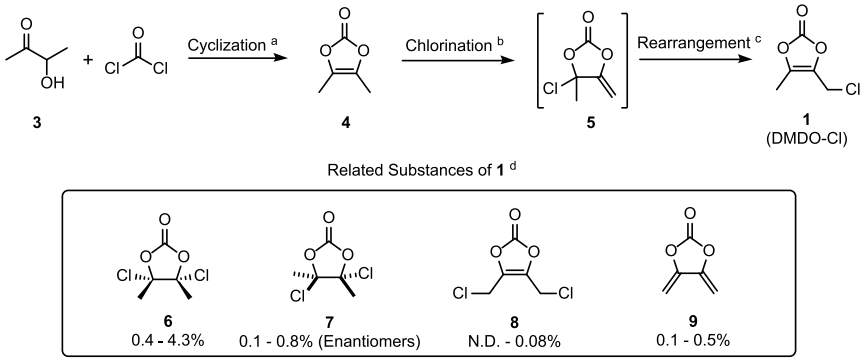


Figure 1. Launched active pharmaceutical ingredient containing the DMDO functional group.

Scheme 1. Current Manufacture Route of DMDO-Cl and Related Substances



[a]Cyclization partner could be phosgene ($COCl_2$) or triphosgene ($CO(OCCl_3)_2$). [b]Cl_2, or TCCA, or TCT or $SOCl_2$, or SO_2Cl_2, DCM or DCE. [c]75−90 °C. [d]Detected by GC normalized area percent (area%).

unable to reach a quantitative conversion due to the trend of analyte self-degradation, (ii) some derivatization reactions could easily produce a mixture of mono- and multiple-substituted derivatives, and (iii) lack of guiding references: the main derivatization protocols focus on enhancing the sensitivity and volatility of the analyte rather than obtaining a physically and chemically stable standard substance for assay determination.

Currently, the detection methods for halogenated compounds largely focus on the trace-level analysis of relevant substances within active pharmaceutical ingredients (APIs) due to their potential genetic toxicity.[6−8] These methods include the utilization of highly sensitive LC/GC−MS techniques as well as chemical derivatization to enhance the detection response of halogenated compounds. However, in the assay determination of halogenated compounds, conventional GC methods are inadequate due to the inherent instability of certain halogenated compounds and sometimes the lack of a stable and accessible method for obtaining analytical reference standards. To date, there has been a dearth of precise quantification reports for thermally labile halogenated compounds. Herein, we aim to overcome the challenges associated with quantifying unstable halogenated compounds, exemplifying our research with the representative compound 4-(chloromethyl)-5-methyl-1,3-dioxol-2-one (DMDO-Cl, **1**), as outlined below.

DMDO-Cl is a key raw material widely used to transform the carboxylic acid form into an active prodrug, which enhances oral bioavailability in the field of cardiovascular and antibacterial medication. The formed dimethyl-1,3-dioxol-2-one (DMDO) side chain is prone to hydrolysis and releasing the active form of the prodrug after being absorbed by the digestive tract.[9,10] The structure of a DMDO group has been incorporated into considerable launched small molecular drugs and leading compounds (medoxomil ester or amide), including lenampicillin, prulifloxacin, olmesartan medoxomil, azilsartan medox-

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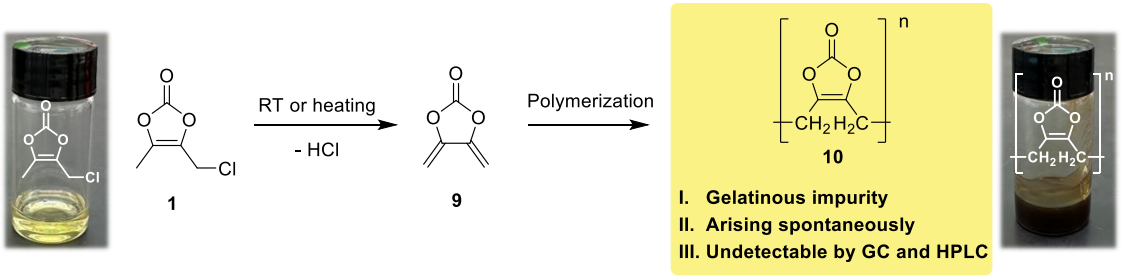
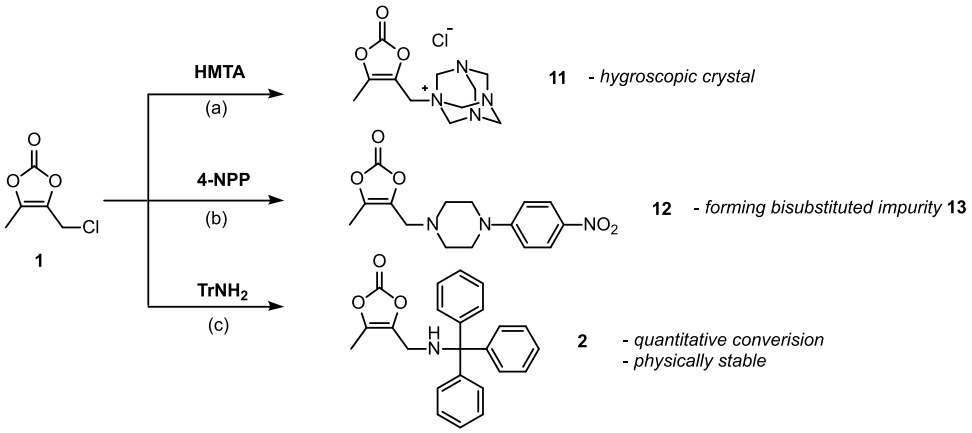


Figure 2. Generation of polymeric impurity **10**.

Scheme 2. Comparison for Three Amine Derivatives of 1



[a]HMTA (1.5 equiv), chloroform, 60 °C. [b]4-NPP (1.1 equiv), K_2CO_3 (1.5 equiv), KI (0.1 equiv), acetone, 20 °C. [c]$TrNH_2$ (1.3 equiv), DIPEA (1.3 equiv), KI (0.1 equiv), DMF, 20 °C.

omil, ceftobiprole medocaril, and faropenem medoxomil, leading to an augmented consumption of its precursor **1** over the past three decades (Figure 1).

Since the first synthesis of the bromide analogue of **1**, efforts have remained focused on the creation of robust reaction conditions and economical isolation processes to improve the quality and minimize production costs. The typical manufacturing route of **1** is outlined in Scheme 1. Starting from 3-hydroxy-2-butanone (**3**), cyclization with phosgene or triphosgene followed by filtration afforded solid product **4**.[11] Various chlorinating reagents including chlorine,[12] trichloroisocyanuric acid (TCCA),[13] trichlorotriazine (TCT),[11] *N*-chloro-succinimide (NCS),[12] and sulfuryl chloride (SO_2Cl_2)[14,15] could chlorinate the conjugate form of **4** to give **5** as an unstable intermediate, which easily underwent allylic rearrangement upon heating, followed by molecular distillation[16,17] to give final product **1**, along with other related substances (lower half of Scheme 1; the content of related substances was retrieved from one of the suppliers).

The quality of compound **1** is chiefly determined by its shelf life stability, which is unfortunately deficient. During the course of transportation and storage, **1** undergoes gradual decomposition at ambient temperature, with this reaction rate being accelerated by increasing the temperature. Consequently, diene **9** is generated, which can undergo spontaneous polymerization and yield the polymeric impurity **10**[18] (Figure 2). Notably, **10** is undetectable by gas chromatography (GC) and its concentration rises over time. Empirical evidence obtained from batch-to-batch evaluations indicated that the purity of **1** could be >96 Area% despite months of long-term storage, although the color darkens and fluidity deteriorates. The elimination-polymerization decomposition of **1** led to inaccurate analysis by GC normalized area%. Additionally, the instability of **1** means that no standard substance is commercially available, which consequently makes it challenging to undertake quantitative analytical determination of this compound.

Therefore, there is an urgent need to develop a fast, reliable, and precise analytical method for the quantitative determination of **1**. To address the issue of standard substance availability for oily compound **1**, it was proposed that it be converted into several solid derivatives. Given that the primary halide group of **1** is prone to be attacked by amines, three derivatized structures were designed—4-nitrophenyl piperazine (4-NPP), hexamethylenetetramine (HMTA), and triphenylmethanamine ($TrNH_2$). The ideal derivatives should meet the following criteria: (i) highly pure and stable, (ii) nonhygroscopic crystals, (iii) high reactivity and selectivity under derivatization conditions, and (iv) preferably bearing chromophores for high-performance liquid chromatography-ultraviolet (HPLC-UV) detection; otherwise, a charged aerosol detection would be needed. Thus, this article compares the reactivity and physical properties of the three aforementioned derivatives and selects the most compliant derivative for analysis method development and validation.

RESULTS AND DISCUSSION

Screening of the Derivatives. The derivative screening aimed to evaluate the conversion from analyte **1** to its corresponding derivatives and their physical properties as standard substances. HMTA was utilized to form ammonium salt **11** under neutral conditions (Scheme 2). Through experimentation, it was found that **11** was a hydroscopic crystal

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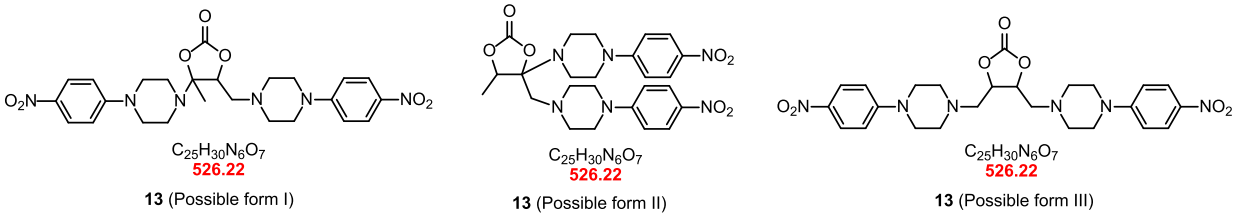
Chart 1. Hypothesized Structure of Impurity 13 Based on LC−MS

Impurity m/z: 527.3 $[M+H]^+$



and did not meet the basic criteria for an ideal standard substance. As a result, attention was turned to reacting **1** with 4-NPP to give tertiary amine **12**, which possessed good crystallinity due to the presence of the p-nitrophenyl moiety (Scheme 2). To our disappointment, a major impurity with over 30 area% in the reaction mixture was observed and assigned as bisubstituted impurity **13** based on LC−MS analysis (see Figure S5). The hypothesized structure of **13** is depicted in Chart 1. Despite additional screening of solvents, bases, and reaction temperatures, controlling the formation of **13** was unsuccessful. The weak acidity of 4-NPP may partially facilitate the nucleophilic addition that leads to the formation of bisubstituted impurity **13**. More importantly, its formation may be attributed to the polarization induced by oxygen atoms, rendering the derivatized DMDO more susceptible to a second nucleophilic attack. Therefore, selectively alkylating the chloromethyl group quantitatively without forming an addition upon the labile DMDO scaffold is quite challenging.[19]

The use of $TrNH_2$ as a sterically hindered primary amino reagent showed promise, offering good selectivity of mono-alkylation under the presence of *N,N*-diisopropylethylamine (DIPEA) and catalytic KI to afford **2**, which is a crystalline solid. It is noteworthy that when a substantial excess of triphenylmethane (10 equiv) is introduced, halogenated compound **1** does not yield bisubstituted products; instead, it exclusively produces monoalkylated product **2**. This satisfying finding bolstered our confidence in employing triphenylmethane as a derivatization reagent. The single crystal X-ray diffractometer monocrystal structure of the triphenylmethanamino (TrNH−) derivative **2** is shown in Figure 3. The atropisomerism of **2** could be attributed to the restricted rotation about the $-CH_2-$ group,

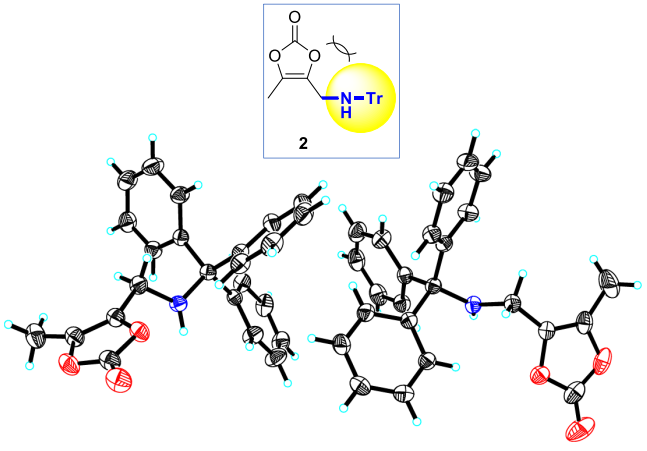


Figure 3. Single crystal X-ray diffractometers of derivative **2** (racemic atropisomers).

which serves as the linker of the DMDO scaffold and the bulky TrNH− group. It is noteworthy that the two structurally similar atropisomers of **2** did not impact the quantitative determination of **1**, which could be utilized as a racemic derivative.

Optimization of Derivatization Conditions. The enabling condition of derivative **2** provided the compound as a derivatized standard (HPLC purity 99.99 area%) to determine the content of **1**. The residual solvents and other volatiles of standard **2** were determined by differential scanning calorimetry-thermal gravimetric analysis (DSC-TGA) with an outcome of <0.05%. The residue on the ignition test confirmed that the ratio of inorganics and ashes was below the threshold of 0.10%. By employing the aforementioned standardization approaches, the suitability of compound **2** as a standard substance was ensured before the derivatization conditions. In order to facilitate the comparison, qNMR tests, which possess broad applicability, were adopted as an alternative method to determine the content of compound **1**. The results are presented in Table 1, in which the assay of **1** was 96.36 ± 0.63% with an RSD of 0.65% through three parallel experiments and calculations.

Table 1. qNMR Results for Quantitative Analysis of 1

analyte[a]	assay (%)	mean ± SD (%)	RSD
1	97.08	96.36 ± 0.63	0.65%
	95.92		
	96.09		

[a]Thymol (IUPAC name: 5-Methyl-2-(propan-2-yl)phenol) was used as a qNMR internal standard in $CDCl_3$. Comparing the integral of the peak (4.30−4.35 ppm, s, $-CH_2-$ of **1**) and of the peak (3.10−3.25 ppm, dt, −CH− of thymol).

To identify an optimal and user-friendly method, three reaction solvents [*N,N*-dimethylformamide (DMF), acetonitrile (MeCN), and 2-butanone (MEK)] were screened along with the reaction temperature (25, 35, and 45 °C). The precolumn standard derivatization procedure for conditions optimization was depicted as a flowchart, as shown in Figure 4. The derivatization material was prepared in centrifuge tubes by accurate weighing, followed by a 5 h reaction process in a temperature-controlled water bath. Subsequently, sampling and volume fixing (diluted with MeCN) were carried out prior to HPLC analysis, which set a comparison between the standard solution of **2** and the derivatization reaction mixture containing **2**. The outcome of the proposed derivatization method was then compared to the qNMR results to calculate the recovery rate under different conditions:

$$\text{Assay of } \mathbf{1} = \frac{m_1}{m_2} \times \frac{A_2}{A_1} \times \frac{V_1}{V_2} \times \frac{M_1}{M_2} \times P \times 100\%$$

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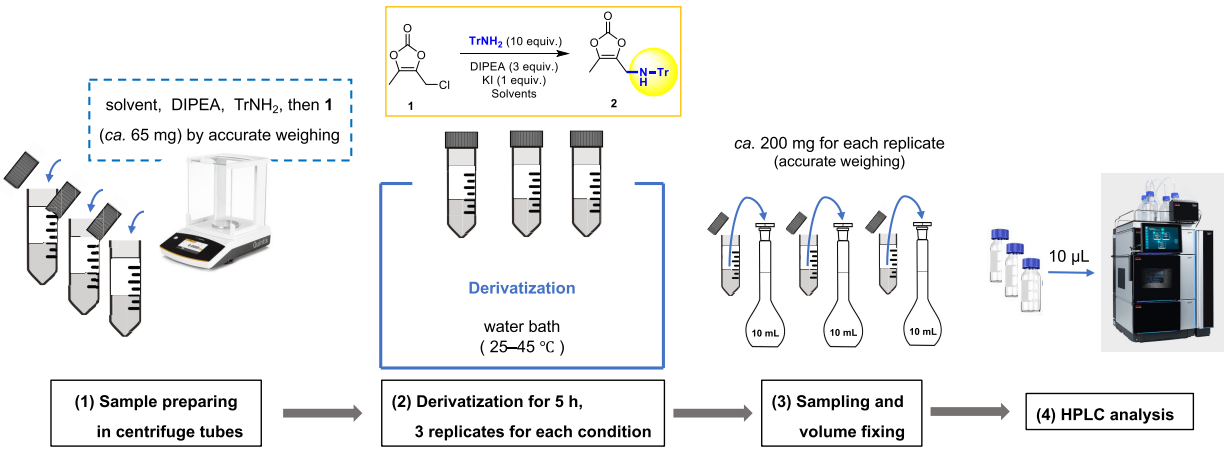

Figure 4. Standard derivatization method for condition optimization.

M_1: molecular mass of **1**, M_2: molecular mass of **2**, V_1: dilution volume of the sample solution of **1**, V_2: dilution volume of the external standard solution of **2**, $A1$: peak area integral of the sample solution of **1**, A_2: peak area integral of the external standard solution of **2**, m_1: mass of **1**, m_2: mass of **2**, and P: purity of the standard substance **2** as mass fraction.

To ensure safety, the temperature range was not expanded beyond the given scope, as the implementation of thermotolerant glass flasks and reflux equipment would be necessary in that case.

To achieve rapid conversion, a substantial amount of derivatizing reagent was utilized. Specifically, 3 equiv of DIPEA, 10 equiv of $TrNH_2$, and 1 equiv of KI (based on analyte **1**) were necessary. As anticipated, the outcome depicted in Figure 5 exhibited significant variations across different

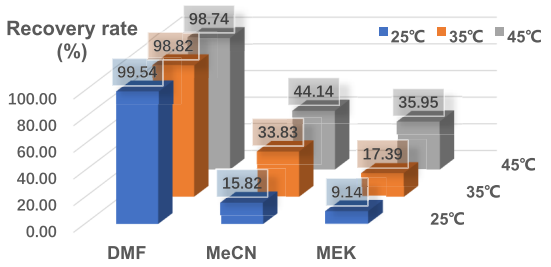

Figure 5. Screening of solvent and temperature.

solvents, which is one of the characteristics of a typical SN_2 *N*-alkylation reaction. When DMF was employed as the solvent, the recovery rate consistently exceeded 98.5% at 25−45 °C. In contrast, a considerable decline in the recovery rate was observed when using MeCN (15.8−44.1%) and 2-butanone (MEK, 9.1−36.0%). Notably, increasing the temperature of the reaction in DMF slightly diminishes the recovery rate (−0.7%), indicating that the ambient temperature could accomplish a complete conversion (>99.5%) and an elevated temperature may lead to trace decomposition. Therefore, DMF was deemed the optimal reaction solvent. The reaction time was determined by tracking the residual amount of substrate **1** over time in the reaction mixture by using GC analysis (external standard). The unreacted **1** at reaction times of 1.5, 3, and 5 h were 5.93, 0.08, and 0.01%, respectively, indicating that derivatization can reach completion within 5 h under the established conditions. Ultimately, the reaction parameters were set as follows: (i) a reaction temperature of 25 °C, (ii) a concentration of analyte **1** at 20 mg/mL (DMF as the solvent), and (iii) a total reaction time of 5 h.

Method Validation. The proposed derivatization method was subjected to validation to demonstrate its feasibility under optimal conditions with respect to specificity, linearity, precision, accuracy, and stability. These validation parameters were assessed according to the guidelines established by the International Council for Harmonisation (ICH)[20] and the Chinese Pharmacopoeia.[21]

Specificity of the Method. The specificity was evaluated by analyzing the chromatograms of derivatization reagents, the target derivatization product **2**, and the potential degradation impurity triphenylmethanol. The results, as shown in Figure 6, revealed that analyte **2** was well separated from the derivatization reagents and triphenylmethanol. The chromatogram at 230 nm exhibited a smooth baseline, and no interference of the matrix was observed.

Similarly, compounds **6**, **7**, and **8**, which are dihalogenated derivatives of compound **1**, may undergo derivatization under the given conditions to yield the corresponding triphenylmethylamine derivatives. Therefore, the derivatization process of these related substances has also been carried out, excluding any potential interference they might introduce to the quantification of compound **1** under the developed HPLC condition, as depicted in Figure 7. These findings indicate high specificity for the proposed method.

It is worth mentioning that the three related substances mentioned above (**6**, **7**, and **8**) are difficult to obtain and require enrichment of the impurity mixture generated during the supplier's large-scale production process, followed by multiple separations. So far, there have been no reported SCXRD structure confirmations for the determination of the absolute configuration of the *cis*- and *trans*-dihalogenated impurities **6** and **7**, which possess two chiral centers with nearly identical ^1H NMR and MS data, suggesting that they are stereoisomers with the same molecular skeleton. Therefore, the absolute configurations of these two compounds remain questionable. Through our efforts in cultivating single crystals and conducting SCXRD testing for those proposed stereoisomers, the absolute configurations of dihalogenated **6** and **7** were validated, as the adjacent chiral centers in both compounds are configured as *cis* (*R*, *S*) and *trans* (*R*, *R* or *S*, *S*) in the single crystals, respectively. In summary, the structures of epimers **6** and **7** have been conclusively confirmed for the first time through SCXRD

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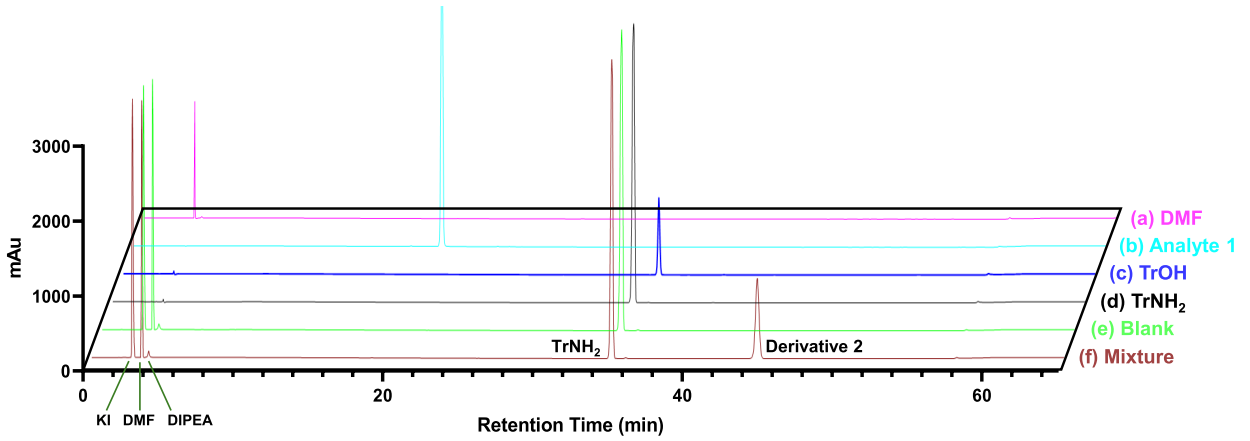

Figure 6. HPLC chromatograms of **1** derivatized by triphenylmethanamine. Each curve represents the following: (a) the reaction solvent DMF, (b) the analyte **1**, (c) the potential degradation impurity triphenylmethanol, (d) the derivatization partner triphenylmethanamine, (e) the blank derivatization condition without analyte **1** (DMF/KI/DIPA/$TrNH_2$), and (f) the sample of the derivatization mixture.

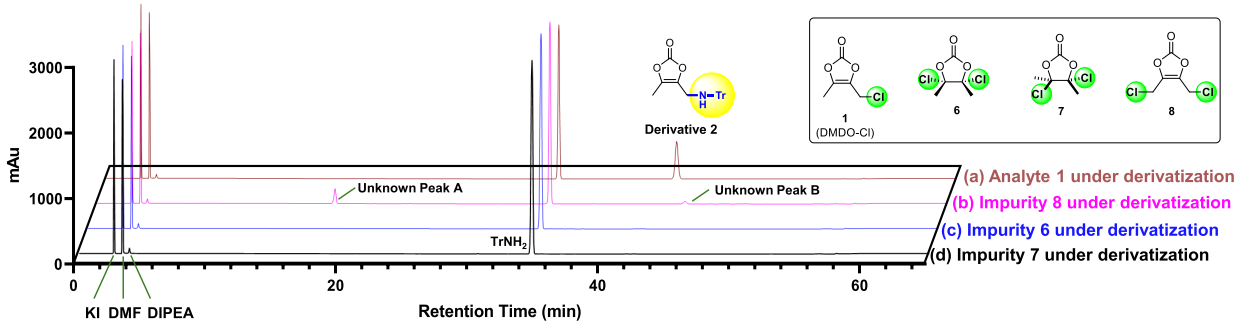

Figure 7. HPLC chromatograms of related substances **6**, **7**, and **8** derivatized by triphenylmethanamine. Each curve represents the following: (a) the derivatization mixture of analyte **1**, (b) the derivatization mixture of impurity **8**, (c) the derivatization mixture of impurity **6**, and (d) the derivatization mixture of impurity **7**.

analysis, which serves as a crucial complement to the results obtained from ^{1}H NMR and MS. (for detailed NMR, mass spectrometry, and SCXRD details of the abovementioned impurities, please refer to the Supporting Information).

Linearity and Stability. The linearity was evaluated by carrying out the derivatization from **1** to **2** at five concentration levels. Then the calibration plots were constructed. The calibration curve was found to be linear over the range of 0.126−0.378 mg/mL for **1**. The linear regression fit, which was 1.0000, was above 0.999 (Table 2). Additionally, the solution

Table 2. Validation of the Method for the Quantitative Determination of 1[a]

analyte	regression equation	linear range (mg/mL)	r	precision RSD%	stability (%)
1	$y = 759.3554x - 0.4130$	0.126−0.378	1.0000	0.74	99.8−100.2

[a] x−concentration (mg/mL), y−peak area, r − correlation coefficient of regression equation.

stability of **2** was also acceptable over a period of 24 h at ambient temperature, with the ratio of peak area to 0 h ranging from 99.8 to 100.2% (Table 2). These results indicate that the method was highly accurate and stable for the determination of **1**.

Intraday and Interday Precision. The intraday precision was assessed by injecting six replicates of derivatization solutions with the analyte level set at 100%. The assay of **1** was in the range of 95.32−97.36% within and an RSD of 0.74%. The evaluation of interday precision was conducted by performing six consecutive injections of the derivatization solutions containing the analyte at a level set to 100%. This process was carried out using two distinct instruments on separate dates. The assay of **1** exhibited a satisfactory range of 94.80−96.71% with an RSD of 0.87% (Table 3). These results indicate the accuracy and precision of the method, thus supporting its reliability.

Table 3. Intraday and Interday Precision

	assay (%)	RSD% (n = 6)	RSD% (n = 12)
intraday	95.32−97.36	0.74	0.77
interday	94.80−96.71	0.87	

Recovery. To investigate recovery, three spiking levels (60, 100, and 140%) of **1** were used. The mean recoveries were calculated as recovery (%) = 100 × C/C_s, where C represents the measured concentration and C_s represents the spiked concentration. The results are summarized in Table 4, with good recoveries in the range of 98.3−100.9% achieved for these analytes with an RSD% of 1.0%.

In general, the method was found to be specific, precise, and accurate for quantifying **2** to calculate the assay of **1**.

CONCLUSIONS

This article presents a novel chemical derivatization-chromatographic detection method for quantitative analysis of the DMDO prodrug precursor **1** (DMDO-Cl). This approach

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Table 4. Recovery Data for the Quantitative Determination of 1

analyte	spiked concentration (mg/mL)	measured concentration (mg/mL)	recovery (%)	RSD%
1	0.153−0.175	0.153−0.172	98.3−100.6	1.0
	0.250−0.290	0.252−0.287	99.0−100.8	
	0.344−0.371	0.347−0.367	98.9−100.9	

effectively addresses two significant challenges encountered in traditional GC or headspace-GC analysis of normalized area percentage of **1**: (i) the inability to accurately reflect the content of **1** due to the potential generation of polymeric impurity **10 and** (ii) the absence of a commercially available standard substance of **1** with reliable nominal content for external standardization. By employing a quantitative conversion of **1** to **2** through the developed triphenylmethanamino-alkylation derivatization, the authentic content of **1** could be directly calculated based on the analysis of the external standard of **2**, which can be easily synthesized and purified. The derivatization conditions were screened and optimized, resulting in a conversion rate exceeding 99.5% within a 5 h reaction period under ambient temperature. Method validation demonstrated the reliability of this approach, exhibiting high specificity, good linearity, good precision, and accuracy. For the first time, this paper unveiled the potential of applications of triphenylmethanamine as an analytical derivatization reagent to improve detection sensitivity and physical crystallinity of the derivatized analyte.

The precolumn derivatization protocol provides a simpler and more cost-effective alternative to the general qNMR detection, whereas a slight decrease in specificity could be observed in the latter method when levels of impurity in analyte **1** were raised. All reagents and apparatus employed were readily available and inexpensive, thereby facilitating widespread application scenarios of this approach in the future. Nevertheless, the limitation of this study lies in the fact that it addresses only the challenge of quantifying the assay of chemically reactive DMDO-Cl through derivatization, while the detection challenge of polymeric impurity **10** remains unresolved. On the other hand, the design of this method was initially intended for accurate quantification of DMDO-Cl as the starting material in APIs' process studies, and its application for the detection of trace amounts of DMDO-Cl as a halogenated compound with genotoxic alerting structures in different APIs is still to be developed.

The process of this study showcased a combination of analytical chemistry and chemical reaction design. It demonstrates that the quantification of a certain class of compounds with poor stability, lack of reference substances, and high reactivity can be addressed by identifying a chemically reactive reaction with quantitative transformation, mild conditions, and the production of crystalline products with good stability. In this study, the reported triphenylmethanamino-derivatization method could meet all of the criteria above, holding potential for further development in future analytical applications, particularly for the determination of challenging content levels in other halogenated compounds due to their inherent chemical instability. To ensure quantitative transformation of the reaction, at least two comparative measurement methods should be employed and the potential occurrence of side reactions needs to be assessed to avoid any impact on quantitative analysis.

EXPERIMENTAL SECTION

Instrumentation and Chromatographic Conditions. The experiments were performed on a Thermo Vanquish Core series HPLC system (Thermo Fisher Scientific Inc., CA, USA) with a DAD detector. An online workstation (Chromeleon) was used to control the system and data acquisition. Weighing of chemicals and reagents was performed on a Sartorius Quintix SQP laboratory balance (Sartorius AG, Goettingen, Germany). A Thermo Acclaim C18 column (250 mm × 4.6 mm, 5 μm) was used for the analysis. The mobile phase was composed of acetonitrile (mobile phase A) and a 10 mM ammonium acetate solution (mobile phase B). The chromatographic separation was achieved using the following gradient elution: 0−5 min, 10% A; 5−30 min, ramping from 10 to 70% A; 30−55 min, 70% A, with a constant flow rate of 1.0 mL/min. The column temperature was maintained at 30 °C. The typical injection volume was 10 μL.

qNMR Experiments. qNMR was performed on a Bruker BioSpin instrument (Billerica, MA, USA) operating at a proton resonance frequency of 400.13 MHz. The relaxation delay was 60 s, the acquisition time was 4.0894 s, and 16 scans were accumulated for each sample.

Chemicals and Reagents. Ammonium acetate (HPLC grade) and *N*,*N*-dimethylformamide (DMF, 99.5%GC, AR) were purchased from Aladdin Scientific (Shanghai, China). Acetonitrile for mobile phase (HPLC grade) and potassium iodide (KI, 99.0%) were purchased from Adamas-beta (Shanghai, China). *N*,*N*-diisopropylethylamine (DIPEA, 99.5%) was purchased from Energy-Chemical (Shanghai, China). Triphenylmethanamine (98.0%) was purchased from Bidepharm Ltd. (Shanghai, China). Thymol (analytical standard, 99%) was purchased from Macklin Biochemical Co. (Shanghai, China). 4-(Chloromethyl)-5-methyl-1,3-dioxol-2-one (DMDO-Cl, **1**) was purchased from Huanggang Luban Pharmaceutical Co.

Sample Preparations and Derivatization Procedure.

1. Preparation of stock solutions: Separate stock solutions of KI, DIPEA, and triphenylmethanamine were freshly prepared in DMF for the derivatization experiments. The 80 mg/mL KI solution was prepared by accurately weighing and volume fixing ca. 8 g of KI in 100 mL of DMF. Similarly, the 200 mg/mL DIPEA solution was prepared by accurately weighing and volume fixing ca. 20 g of DIPEA in 100 mL of DMF. The 400 mg/mL triphenylmethanamine ($TrNH_2$) solution was prepared by accurately weighing and volume fixing ca. 100 g of $TrNH_2$ in 250 mL of DMF.
2. Preparation of the external standard solution of derivative **2**: Approximately 10 mg of compound **2** was accurately weighed and dissolved in 10 mL of acetonitrile by volume fixing.
3. Derivatization: In a 5 mL volumetric flask, 1 mL of the KI stock solution and 1 mL of the DIPEA solution were accurately measured and combined. Approximately 65 mg of **1** was then accurately weighed and added to the mixture. The resulting mixture was diluted with the $TrNH_2$ stock solution by volume fixing to a final volume of 5 mL. It was then vortexed and left at room temperature (25 °C) for 5 h to complete the reaction. After reaction completion, 0.2 mL of the reaction solution was accurately removed and transferred into a 10 mL volumetric flask. The solution was further diluted with

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acetonitrile by volume fixing. The resulting acetonitrile-diluted solution was directly analyzed using an HPLC-UV system, with an injection volume of 10 μL.

Note: The described reaction concentration ensured the formation of a homogeneous clean solution in the DMF-based derivatization reaction. Consequently, when diluting the reaction solution with MeCN, the dilution was performed based on volume (200 μL to 10 mL). It is important to note that other solvents used in the screening experiments, such as MeCN and MEK, resulted in slightly turbid mixtures at temperatures ranging from 25−45 °C due to the reduced solubility of the derivatization materials. As a result, the sampling of the reaction mixture was performed by accurate weighing after vortexing. Subsequently, the turbid sample was diluted with MeCN (200 μL to 10 mL) to obtain a homogeneous clean solution suitable for HPLC injection.

Synthesis of 4-Methyl-5-((tritylamino)methyl)-1,3-dioxol-2-one (2). A 250 mL three-necked flask was charged with *N,N*-diisopropylethylamine (DIPEA, 50.13 mmol, 1.3 equiv), potassium iodide (KI, 3.86 mmol, 0.1 equiv), triphenylmethanamine (10.01 g, 38.56 mmol, 1 equiv), *N,N*-dimethylformamide (DMF, 60 mL), and 4-(Chloromethyl)-5-methyl-1,3-dioxol-2-one **1** (7.56 g, 50.13 mmol, 1.3 equiv). The resulting mixture was stirred at 65 °C for 24 h. Triphenylmethanamine was completed and converted into **2** by TLC monitoring. The reaction mixture was cooled down to room temperature. Upon stirring, water (60 mL) and ethyl estate (60 mL) were added, and the agitation was maintained for 15 min. The organic layer was separated, and the aqueous layer was extracted with ethyl estate (40 mL). The organic layer was combined, washed with saturated aqueous NaCl solution (2 × 40 mL), dried over Na_2SO_4, and condensed in vacuo to give a brown oil. The brown oil was dissolved with t-BME (40 mL) and decolored by silica gel (10 g, 200−300 mesh) prior to filtration. Evaporation in vacuum gave 6.93 g of claret oily residue as a crude product. To the residual was added ethanol (20 mL), and the resulting slurry was agitated at room temperature for 10 min. After solidification of the white crystals, the slurry was heated to reflux for 10 min, cooled to room temperature, held for 2 h, and then filtered. The cake was washed with ethanol (10 mL) and deliquored to give 3.95 g of wet off-white crystalline powder. Recrystallization by diisopropyl ether−acetone (16 mL, diisopropyl ether−acetone 5:3) and drying under vacuum (45 °C) gave 2.835 g of colorless crystalline powder (16.6% yield, HPLC purity: 99.99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.42 (dt, J = 8.2, 1.3 Hz, 6H), 7.32 (dd, J = 8.4, 6.7 Hz, 6H), 7.26−7.17 (m, 3H), 3.49 (t, J = 7.8 Hz, 1H), 2.93 (d, J = 7.8 Hz, 2H), 1.95 (s, 3H). Residue on ignition test: the ratio of inorganics and ashes below the threshold of 0.10%. DSC-TGA test: volatiles below the threshold of 0.05%.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c09982.

qNMR spectra and results of **1**, ^{1}H NMR, SCXRD unit cell of **2**, LC−MS spectra of derivatization reaction forming **12**, and validation tables with complete data (PDF)

SCXRD data of compound **6** (CIF)

SCXRD data of compound **7** (CIF)

SCXRD data of compound **2** (CIF)

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Author Contributions

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Notes

The authors declare no competing financial interest.

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