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# Quantification of alcohols, diols and glycerol in fermentation with an instantaneous derivatization using trichloroacetyl isocyanante via liquid chromatography-massspectrometry

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ABSTRACT

A sensitive LC–MS/MS method was established to quantify diols and glycerol in fermentation broth using trichloroacetyl isocyanate as instantaneous derivatization reagent for monitoring the production of 1,3-propanediol and 2,3-butanediol from the biodiesel biorefinery process. Due to the derivatization reaction was very quickly at room temperature, only 1 min was needed for the reaction process. In addition, both extraction of analytes and evaporation of water were not employed in the analytical procedure. Furthermore, the isotope of chlorine was beneficial for understanding of the secondary mass spectrum and avoiding false positive results. Therefore, much more accurate results of diols and glycerol concentration in fermentation could be obtained even at very low levels for the evaluation of microbial metabolism pathway modification.



## 1. Introduction

Distinct from traditional chemical engineering which was labeled as high pollution, biodiesel biorefinery was an environment friendly technology to obtain target products by fermentation of microbial [1]. Regarding to the increasing consumption of 1,3-propanediol, the monomer for polytrimethylene terephthalate (PTT), bio-synthesis from glycerol was a hot topic in the biological engineering research [2]. In order to promote the production of target products, modification of the carbon metabolism pathways was the principal method [3]. Quantification of diols in the fermentation broth was significant in the evaluation of pathway modification.

GC–MS was the principle method for the quantification of diols [4–8], however, it was difficult to extract diols from fermentation broth to organic solution due to its strong hydrophilic property. Although solid phase extraction (SPE) had been employed for the sample preparation procedure of bronopol [9], separation of 1,3-propanedio from fermentation via SPE had not been reported yet. HPLC was a substitution quantification method for alcohol quantification [10,11]. However, its limit of detection was not satisfied for a new metabolism pathway evaluation when the 1,3-propanedio yield was very low.

Derivatization procedure was essential for alcohol analysis by LC—MS to elevate the ionization efficiency under ESI mode, meanwhile, increase the analyte molecular weight to improve the detection limit and improve the structure elucidatoin [12]. Acyl chloride and sulfonyl chloride were the common reagent for hydoxyl derivatization, such as benzoyl chloride [13,14], pentafluorobenzoyl [15], dabsyl chloride [16], pyridine-3-sulfonyl chloride hydrochloride [17], dansyl chloride [18]. Other kinds of reagents had also been synthesized for alcohol derivatization, such as 2-sulfobenzoic anhydride [19] and *S*-pentafluorophenyl tris(2,4,6-trimethoxyphenyl)phosphonium acetate bromide [20]. Regarding to vigorous reaction conditions of heating and more than 20 min were usually required for derivatization using reagents mentioned above, ethyl chloroformate was used for the derivatization of phenol and amino group, which could be accomplished within 3 min under mild condition [21]. However,

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**Table 1**
Multiple reaction monitoring parameters and retention times.

| Compound name | Precursor ion | Product ion | Collision Energy | Retention time (min) |
|---|---|---|---|---|
| Ethanol | 253 | 236, 208 | 10 | 3.15 |
| ethanediol | 456 | 234, 232 | 15 | 4.67 |
| *n*-butanol | 281 | 208, 146 | 15 | 5.03 |
| 1,3-propanediol | 470 | 248, 246 | 15 | 5.03 |
| 2,3-butanediol | 484 | 262, 260 | 15 | 5.40 |
| Glycerol | 673 | 451, 449 | 20 | 5.73 |

ethyl chloroformate was not suitable for alcohol's derivatization.

In this work, trichloroacetyl isocyanate was used as derivatation reagent for LC–MS/MS analysis of diols and glycerol in fermentation broth. Furthermore, the isotope of chlorine could be utilized to avoid false positive results. The method reported here was convenient especially for aqua samples, and the LOQ were about 0.5 ng/mL for monobasic alcohol and diols, 5 ng/mL for glycerol.

## 2. Materials and methods

### 2.1. Materials

1,3-propanediol and 2,3-butanediol were purchased from Tokyo Chemical Industry Co. Ltd (Tokyo, Japan). Trichloroacetyl isocyanate and acetonitrile (HPLC grade, ACN) were purchased from Sigma-Aldrich Chemical Co. (ST. Loius, MO, USA). Glycerol, ethanediol, ethanol, n-butanol, ammonium acetate ($NH_4AC$) and acetic acid (HAC) were purchased from Beijing Chemical Works (Beijing, China).

### 2.2. Apparatus

The analytical system consisted of 1290 infinity II HPLC (Agilent) coupled with 6495 mass spectrometry (Agilent). Chromatographic separation was performed on an Agilent zorbax eclipse plus C18 column (3.0*50 mm, 1.8 μm), maintained at 40 °C. The mobile phase consisted of (A) 40 mM $NH_4AC$ with 0.5% acetic acid, and (B) ACN. The flow rate was 0.6 mL/min. Separation started with 15% B, maintained for 2 min, increased at 50% for 3 min, maintained for 4 min. The total run time was 9 min.

Determination was done using an ESI source in the positive mode. Setting were set as follows: gas temperature was 200 °C, gas flow was 18 L/min, nebulizer was 35 psi, sheath gas temperature was 200 °C, sheath gas flow was 11 L/min, capillary was 3000 V and the nozzle voltage was 1500 V. Multiple reaction monitoring (MRM) parameters and retention times are presented in Table 1.

### 2.3. Fermentation procedure

All flask cultures were performed in KP medium containing (per liter): 1.3 g $KH_2PO_4$, 3.4 g $K_2HPO_4{\cdot}3H_2O$, 0.2 g $MgSO_4{\cdot}7H_2O$, 3.0 g $(NH_4)_2SO_4$, 20 g glycerol, 10 g glucose, 1 g yeast extract, and 5 mL trace elements. Trace elements contained (per liter): 2.5 g $CoCl_2{\cdot}6H_2O$, 15 g $MnCl_2{\cdot}4H_2O$, 2.1 g $Na_2MoO_4{\cdot}2H_2O$, 100.8 g Fe (II) citrate. The final pH of KP medium was adjusted to 7.0 by 4 mol/L KOH.

The *K. pneumoniae* cells were inoculated into 4 ml LB medium and cultivated overnight in a rotary shaker at 37 °C, 160 rpm. Then 500 μl cultures were transferred into 50 ml KP medium in 100 ml Erlenmeyer flask for flask cultures. All flask cultures were carried out for 24 h.

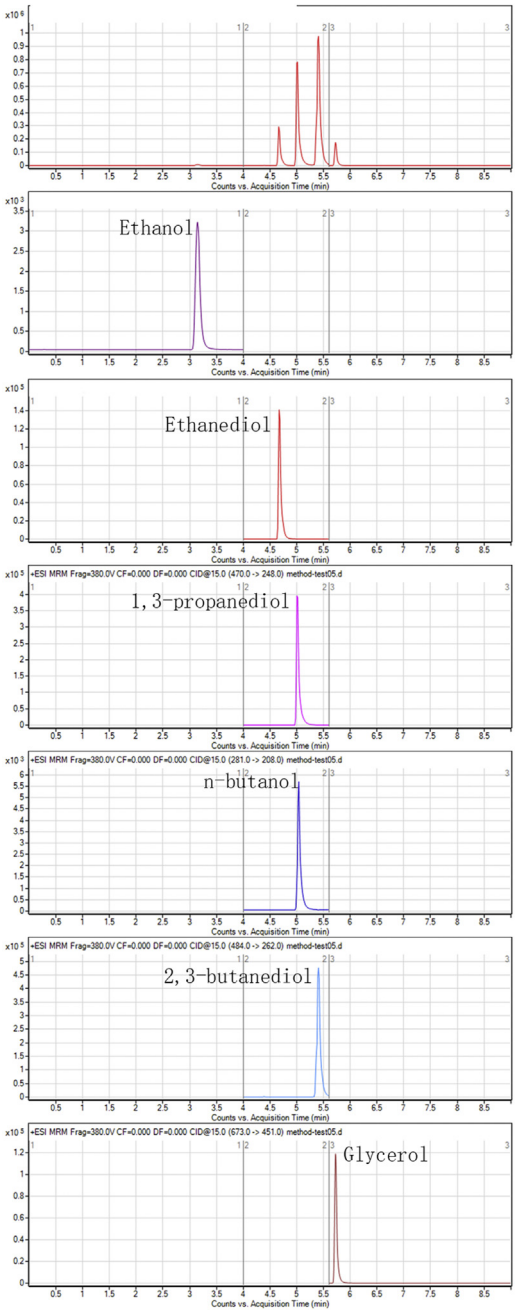


**Fig. 2.** LC–MS/MS chromatography of analytes.

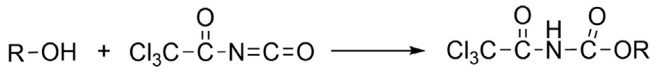


**Fig. 1.** The reaction mechanism of trichloroacetyl isocyanate with alcohols.

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