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Chromatographic fingerprints of industrial toluic acids established for their quality control

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Abstract

The chromatographic fingerprints of industrial *o*-toluic acid, *m*-toluic acid and *p*-toluic acid have been established by HPLC-UV detection according to their impurity groups. HPLC separation of all relative substances involved in the groups was developed on a Kromasil C_{18} column by using methanol–water–NH_4Ac-HAc buffer (100 mM, pH 4.70) 15/65/20 (v/v/v) as the mobile phase at a flow rate of 1.5 mL/min, and detection was operated by UV adsorption at a wavelength of 254 nm. The ultraviolet spectra corresponding to each chromatographic peak were also recorded for further identification of all components. Whether the limits of relative impurities residues in a toluic acid product are qualified or not can be intuitively estimated by analyzing its chromatogram with comparison to the fingerprint. This protocol has successfully provided some Chinese manufacturers with a simple and feasible method for quality control of toluic acids for industrial use.

Keywords: Chromatographic fingerprint; Fine chemical intermediate; *o*-Toluic acid; *m*-Toluic acid; *p*-Toluic acid; HPLC; Ultraviolet spectrum

1. Introduction

Chromatographic fingerprint analysis is a rational approach for quality assessment of natural products such as herbal medicine, etc., and the industrial products of natural origins such as wine, edible oil and petroleum, etc., each of which contains many compounds that may be relevant to the medical, nutrient or other putative activity [1–4]. A chromatographic fingerprint is, in practice, a chromatographic pattern of extract of some common chemical components. This chromatographic profile is featured by the fundamental attributions of "integrity" and "fuzziness" or "sameness" and "difference" so as to chemically represent the samples investigated. This strategy has been playing more and more important role in traditional Chinese medicine (TCM) quality control [1]. In the last two to three decades, China has been becoming a main exporter of fine chemical intermediate (FCI) in the world. FCI products require strict quality control to enter the international market [5,6]. Analytical methods are needed not only to verify the product purity but also to identify the nature of related substances existed, and are of primary importance in fine chemical industry [7,8]. Based on the current situation of analysis and testing of fine chemicals including intermediates in China, we put forward the conception of FCI chromatographic fingerprint and suggested that the fingerprint should be established by using the experience of TCM [6]. The significance of the chromatographic fingerprint for research, development, manufacture and trade of FCIs was presented. The feasibility of establishment of FCI chromatographic fingerprints was discussed, and moreover, the general procedures and the essential techniques for the strategy were introduced in detail [6]. Without doubt, HPLC is one of the dominating techniques for this purpose. As exemplified with chromatographic fingerprints of *o*-toluic acid (OTA), *m*-toluic acid (MTA) and *p*-toluic acid (PTA) for industrial use, which have been built by HPLC combined with spectrophotometric detection, these fingerprints have been successfully applicable for the routine quality control in some chemical plants manufacturing the three products.

OTA, MTA and PTA are all fundamental organic synthetic and fine chemical intermediates, and extensively used in pesticides, medicines, dyestuff, and other fine chemicals. Although there are many ways to obtain these three chemicals, the preparation is mainly based on the partial oxidation of *o*-, *m*- and

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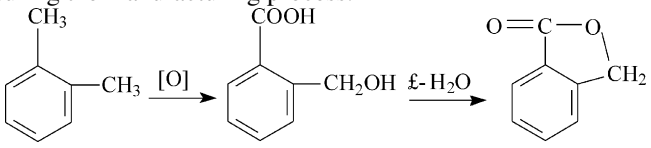
p-xylene, respectively, using different oxidants and catalysts, etc. under certain conditions. So *o*-phthalic acid (OPA), *m*-phthalic acid (MPA) and *p*-phthalic acid (PPA) would easily be the byproducts of industrial OTA, MTA and PTA, respectively, originating from the excessive oxidation of *o*-, *m*- and *p*-xylene. For OTA product, the formation of an additional impurity phthalide (PA) may occur owing to the under-mentioned reaction. Because most of xylenes as the input materials of toluic acids contain more or less amount of toluene and ethylbenzene, benzoic acid (BA) is inevitably present in all final products. In addition, the tiny isomers coexisting in a certain kind of xylene produce corresponding impurities in the reaction. For example, as an isomer of *o*-xylene, *p*-xylene may be turned into PTA and then PPA as impurities in OTA product, such-and-such. To sum up, in the final individual product of toluic acids, BA, OPA, MPA, PPA, even OTA, MTA and PTA, and sometimes PA are all the possible impurities [9–11]. Therefore, the contents of the relative substances in each toluic acid product have to be controlled during the manufacturing process.



Very few reports about the HPLC analysis with regard to industrial toluic acids have been published up to date [12–15]. The analytical methods used only aimed at the purity assay of a single product such as MTA [12], or determination of impurities in a single product such as OTA [13] or PTA [14,15], OPA, MTA, PTA and their related impurities being just regarded as waifs and strays. In fact, all of these compounds are interdependent and interknit. Accordingly, the term "impurity group" was introduced to describe the integrated product profile that is composed of all relative substances existed reasonably and allowably in a FCI product. For instance, BA, OPA, MPA, PPA, MTA, PTA and PA constitute the impurity group of industrial OTA, also, BA, OPA, MPA, PPA, OTA and PTA constitute that of industrial MTA, and BA, OPA, MPA, PPA, OTA and MTA constitute that of industrial PTA, respectively. Therefore, fingerprints of OTA, MTA and PTA products can be drawn based on their impurity groups. In this paper, an HPLC-UV method for the simultaneous determination of eight chemicals specified has been developed. The optimization of the experimental conditions was comprehensively investigated. On the basis of good separation and reliable determination, furthermore, we established the chromatographic fingerprints of OTA, MTA and PTA products, by which quality assessment could be facilitated of industrial toluic acids.

2. Experimental

2.1. Apparatus

Instrumentation for HPLC analysis included a Varian 5060 liquid chromatograph (Varian, Walnut Creek, USA), a Rheodyne 7725i injector valve equipped with a 10-μL loop (Rheodyne, Cotati, USA), a Waters 486 tunable UV absorbance detector (Waters, Milford, USA). Data acquisition and processing was performed on a JS-3050 chromatographic working station (Dalian Johnsson Separation Science and Technology Corporation, Dalian, PRC). The UV spectra of eight chemicals were obtained by Waters Alliance 2695 Separations Module equipped with a vacuum degasser, a quaternary pump, an autosampler and a 996 UV–vis photodiode-array detector (PDA), and Millinium^{32} chromatography manager system (Waters). The latter HPLC system also was used to assess the comparability of chromatographic fingerprints in different apparatus.

2.2. Reagents and chemicals

Reference substances (RS) of OTA, MTA, PTA, OPA, MPA, PPA and PA (99.0% purity) were purchased from Shanghai First Reagent Factory (Shanghai, PRC), Tianjin Second Reagent Factory (Tianjin, PRC), Nanjing Chemical Reagent Factory (Nanjing, PRC) or Taixing Seventh Chemical Factory (Taizhou, PRC). RS of BA (99.5%, purity) was from Shanghai First Reagent Factory. Industrial products of toluic acids were kindly provided by various manufacturers of China. Methanol was HPLC grade (Hanbang Sci & Tech Co. Ltd., Jiangsu, Huai'an, PRC). Water was Wahaha purified water (Wahaha Group Ltd., Hangzhou, PRC).

2.3. HPLC conditions

The column was a Kromasil C_{18}, 150 mm × 4.6 mm i.d., packed with 5 μm particle (Hanbang). The mobile phase was methanol–water–buffer (15/65/20, v/v/v). The buffer was NH_4Ac-HAc (100 mM, pH 4.70). The separation was carried out by isocratic elution with a flow rate of 1.5 mL/min and the column temperature was constantly maintained at 30 °C. The optimum UV wavelength was 254 nm and 10 μL each was applied into HPLC system.

3. Results and discussion

3.1. Development of analytical method

3.1.1. Linear range and detection limit

Individual OTA, MTA, PTA, OPA, MPA, PPA, PA and BA standard stock solutions (1.0 mg/mL) used for calibration purpose were prepared by separately weighting about 25.00 mg of RS into eight 25-mL volumetric flasks and adding methanol to make up to the mark. Then transfer each stock solution 2.50 mL into a 25-mL volumetric flask, mix and add methanol to the volume. The final concentration of each component in this mixed standard solution was 100 μg/mL. Its chromatogram is shown in Fig. 1. Mixed standard solutions at concentration of 0.2–80 μg/mL each component were prepared by serial dilution of the above mixed standard solution with methanol. Linear relationship between peak areas versus concentrations of standards were obtained within the range 0.2–100 μg/mL for PPA, 0.4–100 μg/mL for OPA, and 0.6–100 μg/mL for MPA, BA, PA, OTA, MTA and PTA, respectively. The regression equations with correlation coefficients (r) were