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Determination of small halogenated carboxylic acid residues in drug substances by high performance liquid chromatography-diode array detection following derivatization with nitro-substituted phenylhydrazines

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ABSTRACT

A method for the determination of small halogenated carboxylic acid (HCA) residues in drug substances is urgently needed because of the potential of HCAs for genotoxicity and carcinogenicity in humans. We have now developed a simple method, involving derivatization followed by high performance liquid chromatography-diode array detection (HPLC-DAD), for the determination of six likely residual HCAs (monochloroacetic acid, monobromoacetic acid, dichloroacetic acid, 2-chloropropionic acid, 2-bromopropionic acid and 3-chloropropionic acid) in drug substances. Different nitro-substituted phenylhydrazines (NPHs) derivatization reagents were systematically compared and evaluated. 2-Nitrophenylhydrazine hydrochloride (2-NPH·HCl) was selected as the most suitable choice since its derivatives absorb strongly at 392 nm, a region of the spectrum where most drug substances and impurities absorb very weakly. During the derivatization process, the commonly used catalyst, pyridine, caused rapid dechlorination or chlorine substitution of α-halogenated derivatives. To avoid these unwanted side reactions, a reliable derivatization method that did not use pyridine was developed. Reaction with 2-NPH·HCl using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride as coupling agent in acetonitrile-water (70:30) at room temperature for 2 h gave complete reaction and avoided degradation products. The derivatives were analyzed, without any pretreatment, using gradient HPLC with detection in the near visible region. Organic acids commonly found in drug substances and other impurities did not interfere with the analysis. Good linearity ($r > 0.999$) and low limits of quantitation (0.05–0.12 μg mL^{-1}) were obtained. The mean recoveries were in the range of 80–115% with RSD <5.81% except for 3-CPA in ibuprofen which was 78.5%. The intra- and inter-day precisons were expressed as RSD <1.98% and <4.39%, respectively. Finally, the proposed method was successfully used for the residue determination of the six HCAs in eight drug substance samples.

1. Introduction

Small halogenated carboxylic acids (HCAs) are the main disinfection by-products (DBPs) in drinking water and, because of their genotoxicity and carcinogenicity [1,2], they have received considerable attention from the United States Environmental Protection Agency (USEPA) [3] and the World Health Organization (WHO) [4]. Because of their strong acidity and reactivity, and also because of the distinct biological properties conferred on molecules by the

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introduction of halogen atoms [5], HCAs are often used as alkylation and acylation reagents for the synthesis of medical intermediates, active pharmaceutical ingredients and pesticides in chemical industry [6–13]. In addition to their use in manufacturing processes, HCAs may also be generated by degradation of some raw materials and pharmaceutical products during storage [14]. Low levels of HCA residues may, therefore, be present in final drug substances as potentially genotoxic impurities (GTIs). A threshold of toxicological concern (TTC) for GTIs, based on different dosing durations, has been adopted to estimate their risk as human carcinogens [15–17]. This has raised the need to develop appropriate analytical approaches for monitoring these hazardous compounds in drug substances.

A variety of techniques have been described for the analysis of haloacetic acids (HAAs), which are a subset of HCAs, in water samples. Gas chromatography with electron-capture detection (GC-ECD) [18–21] is the USEPA standard method for detection of HAAs in water samples. Before analysis, the sample must be acidified and the HAAs extracted and derivatized by esterification. High performance liquid chromatography (HPLC), including ion chromatography (IC) [22,23] and reversed-phase liquid chromatography (RP-LC) [24,25], have been combined with electrochemical detection (ECD) or ultraviolet (UV, 210 nm) detection to analyze HAAs in drinking water. These methods usually involve time-consuming pre-concentration and cleanup procedures before analysis to obtain adequate specificity and sensitivity. Hydrophilic interaction chromatography-mass spectrometry (HILIC-MS) [26], GC–MS [27,28], LC–MS [29,30] and IC-MS [31,32] have also been employed for measuring HAAs in environmental water samples and other matrixes. MS-based methods provide excellent specificity and sensitivity but the significant cost of the equipment limits the availability of these methods in a number of laboratories.

Compared with the analysis of water samples, the development of suitable analytical methods for detection of HCAs in drug substances is more challenging. The analysis of complex drug matrixes requires a method with high specificity and sensitivity [16,33] that can eliminate interference from compounds with similar properties to HCAs. Another complication is that, unlike water samples, drug substances can be degraded during the pretreatment process [14] and generate new HCAs, which leads to an overestimation of the amount in the original sample. A suitable analytical method should, therefore, use mild conditions that do not cause drug decomposition and give misleading results. HPLC is now the first choice in most drug quality laboratories. However, for the polar analytes without a strong chromophore and fluorophore, chemical derivatization, combined with HPLC analysis, is a promising method to enhance the separation ability and detection ability, by introducing the specific moiety [33,34]. A laboratory-built automated instrument that uses anion-exchange chromatography to separate HAAs, followed by post-column derivatization and fluorescence detection, has been described for the analysis of water samples [35]. This method may not, however, be suitable for routine drug analysis since it requires a dedicated instrument. Ghassempour et al. [36] have established an analytical approach for the determination of residual amounts of monochloroacetic and dichloroacetic acids in betaine samples based on derivatization with 1-naphthylamine at 90 °C, followed by HPLC with UV detection at 222 nm. Potential weaknesses of this method are that the high temperature used for derivatization may trigger formation of degradation impurities, especially in unstable drugs [37], and detection at 222 nm may result in interference from reagents, other impurities or drug substances [38].

Nitro-substituted phenylhydrazines (NPHs) are well known derivatization reagents for the detection and determination of carboxylic acids [38–45]. Compared with carboxylic acid derivatives prepared using anilines, the corresponding NPH derivatives show preferable absorption characteristics resulting from the strong red-shift effect of the nitro group [39–41]. 2-Nitrophenylhydrazine (2-NPH) [38–43], 3-nitrophenylhydrazine (3-NPH) [44], 4-nitrophenylhydrazine (4-NPH) [45] and 2,4-dinitrophenylhydrazine (2, 4-DNPH) [43] are commercially available. 2-NPH was successfully applied to LC–MS analysis of monofluoroacetic acid (MFAA) in food samples [38]; however, the systematic comparison of the absorption characteristics is a more complex issue than it is presented here.

In the present work, we have developed a simple method for measuring HCA residues in drug substances by derivatization with NPHs under mild conditions, followed by HPLC analysis with DAD detection. During method development, different derivatization reagents and reaction parameters, especially the catalyst, were optimized. To demonstrate the wide applicability of the new method, we examined six analytes, including monochloroacetic acid (MCAA), monobromoacetic acid (MBAA), dichloroacetic acid (DCAA), 2-chloropropionic acid (2-CPA), 2-bromopropionic acid (2-BPA) and 3-chloropropionic acid (3-CPA). This study would be helpful for the routine analysis of HCA residues in drug substances.

2. Experimental

2.1. Chemicals and reagents

MCAA (98%), MBAA (98%), DCAA (98%), 2-CPA (97%), 2-BPA (98%), 3-CPA (97%), vitamin B_6 (98%) and ibuprofen (98%) were purchased from Yunguan Scientific Ltd. (Shanghai, China). 2-NPH·HCl (97%), 3-NPH·HCl (97%), 4-NPH·HCl (98%) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl, 98%) were obtained from Accela (Shanghai, China) and 2, 4-DNPH (99%) was obtained from Aladdin (Shanghai, China). Tadalafil (97%) was kindly supplied by Heron Pharmaceutical Science and Technology Co., Ltd., (Nanjing, China). Chloramphenicol (95%) was prepared by the Department of Medicinal Chemistry, China Pharmaceutical University (Nanjing, China). Metalaxyl (97%) was kindly provided by Xingyun Chemical Factory (Hubei, China). 1-(2-Aminocarbonylbenzofuran-5-yl) piperazine (vilazodone intermediate, 99.2%) was purchased from Abydos Scientific Ltd., (Nanjing, China). 6-Hydroxy-3,4-dihydro-2-quinolinone (cilostazol intermediate, 99.5%) was purchased from Senbeijia Biotechnological Ltd., (Nanjing, China). Chloramphenicol eye drops (8 mL: 20 mg) were purchased from Kangye Pharmaceutical Ltd. (Handan, China). HPLC grade acetonitrile was purchased from Merck (Darmstadt, Germany). Water was purified using a Millipore Milli-Q system (Bedford, MA, USA). All other reagents were of analytical grade and obtained from conventional commercial sources.

2.2. Standard and test solutions

Separate solutions of 2-NPH·HCl, 3-NPH·HCl, 4-NPH·HCl and 2, 4-DNPH at the concentration of 10 mg mL^{-1} were freshly prepared in 80% aqueous acetonitrile for the derivatization experiments. A standard stock solution of EDC·HCl was freshly prepared each day by dissolving EDC·HCl (200 mg) in 60% aqueous acetonitrile (10 mL).

Separate stock solutions containing 1 mg mL^{-1} of each HCA were prepared in 70% aqueous acetonitrile. Before use, a series of mixed standard working solutions were prepared by diluting each stock solution with the same solvent. The working solutions were all stable at room temperature for at least 8 h.

2.3. Derivatization procedure

After taking the appropriate amount of drug substance, EDC·HCl working solution (500 μL) and derivatization solution (500 μL) were placed in a 5 mL vial. After being vortexed for 10 s and diluted