Short communication

Eco-friendly high-throughput screening of cephalosporins impurities: Utilizing 2D-carbon microfiber fractionation system combined with quadrupole time of flight high-resolution mass spectrometer

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Owing to its high sensitivity, selectivity, and accuracy, liquid chromatography coupled with mass spectrometry (LC-MS) is commonly employed to screen, confirm, and quantify impurities in drugs [1]. However, LC-MS has certain drawbacks such as complicated pretreatment steps, long analysis time, large consumption of organic solvents, high maintenance costs, and, most notably, the need for high-pressure pumps and compatible hardware because of the high column backpressure [2]. In this study, a two-dimensional (2D) microscale carbon fiber (CF)/active carbon fiber (ACF) system combined with a quadrupole time-of-flight high-resolution mass spectrometry (2DμCFs-QTOF-HRMS) system was developed to rapid screening impurities in the typical three generations of oral cephalosporins, i.e., cephalexin tablets (CPXTs), cefuroxime axetil tablets (CFATs), and cefixime tablets (CFXTs) (Fig. S1). The "fuzzy chromatographic separation" sample pretreatment method separates complex samples into high-, medium-, and weak-polar fractions for successive detection of MS, achieving effective reduction of the ion suppression effect in electrospray ionization (ESI)-MS and improving the MS detection sensitivity. Compared to high performance liquid chromatography (HPLC)-MS, this method has distinct advantages such as less organic solvent consumption (1.5 mL), shorter separation and analysis times (5 min), more information on impurities, and high reproducibility.

Fig. 1A shows the 2DμCFs system and its overall process of analysis, and the detailed fractionation process is presented in the Supplementary data Part 1. As an example, Fig. 1B displays the total ion current (TIC) chromatograms of the three fractions of CFXTs obtained using 2DμCFs-QTOF-HRMS. The mass spectrum peaks corresponding to the strong-, medium-, and weak-polarity fractions were extracted separately for the component analysis of CFXTs. The ion peaks m/z 454.0495 was identified as $[M+H]^+$ of the principal component cefixime ($C_{16}H_{15}N_5O_7S_2$) by fragments (Fig. S2) and the ion peaks m/z 365.1067 was identified as $[M+Na]^+$ of the auxiliary lactose ($C_{12}H_{22}O_{11}$). Additionally, among numerous impurity peaks, six unknown peaks were putatively identified as impurities I−VI (Figs. S3−S8 and Table S1) [3], with their mass fragmentation pathways explained in the Supplementary data Part 2. The impurities with lower concentrations were detected in the medium- and weak-polar fractions, and the ion suppression from lactose with a high concentration was reduced due to presence in the high-polar fraction. Similarly, principal components and some impurities in CFATs and CPXTs were identified, respectively (Figs. S9−S15 and Tables S2 and S3).

According to the primary component partial change of chemical structure, impurities I−III, IV, V, and VI in CFXTs are speculated to derive from degradation, polymerization, oxidation, and synthetic reaction intermediates, respectively (Fig. S16A). Then for CFATs and CPXTs (Figs. S16B and C), impurities mainly derived from degradation (impurities I−IV in CFATs and I−II in CPXTs) and polymerization (impurities V−VIII in CFATs and III in CPXTs). Particularly, impurities III−VI in CFXTs, III−VIII in CFATs, and I−III in CPXTs were not yet included in the European Pharmacopeia [3].

In short, a total of 17 impurities were putatively identified in these cephalosporins, including six from CFXTs, eight from CFATs, and three from CPXTs. The impurities in different generations of cephalosporins not only originate mainly from degradation rather

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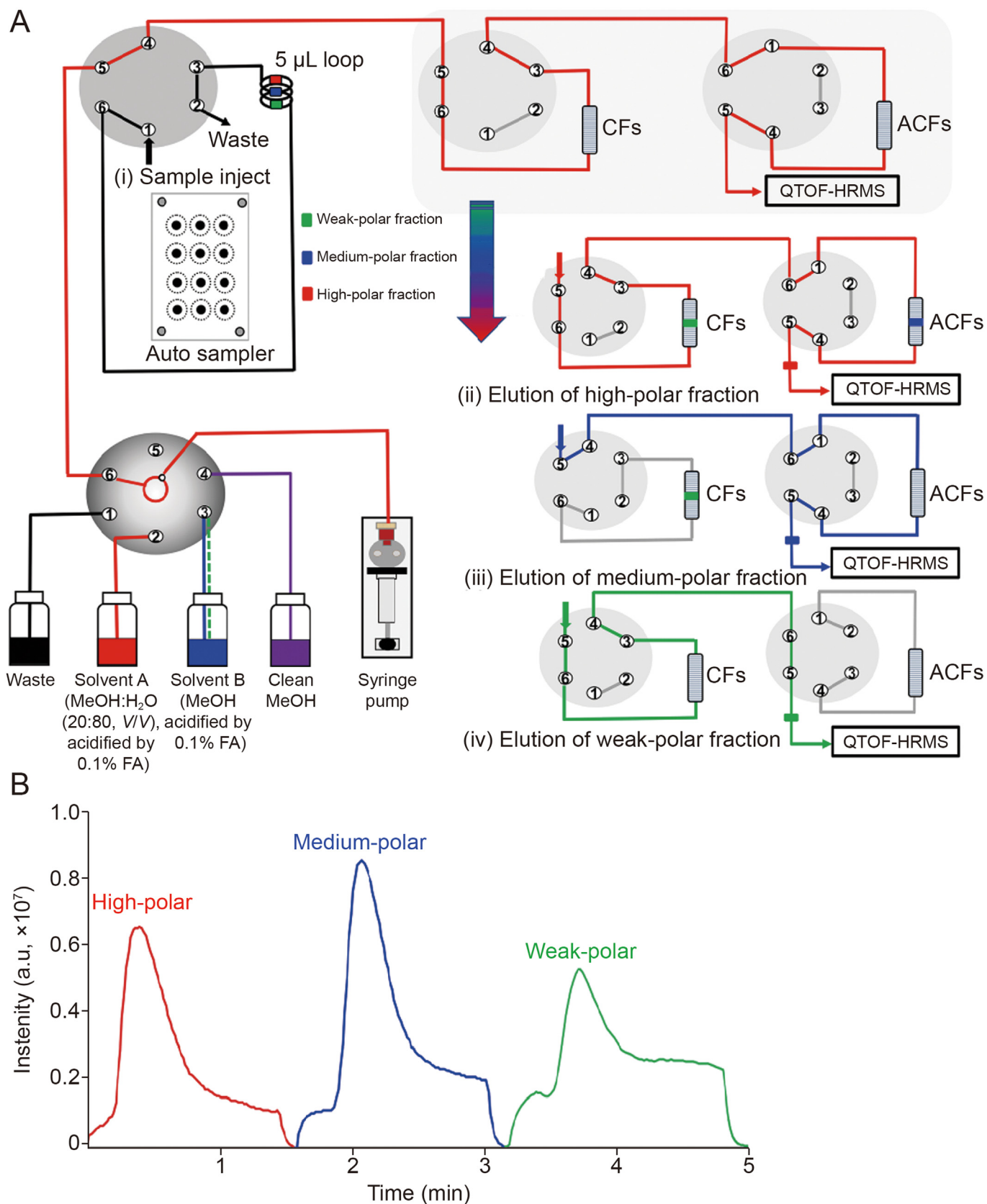[1] Both authors contributed equally to this work.

Fig. 1. (A) Workflow of an on-line two-dimensional microscale carbon fiber/active carbon fiber system combined with quadrupole time-of-flight high-resolution mass spectrometry (2DμCFs-QTOF-HRMS): sample injection (i), elution of the high-polar fraction (ii), elution of the medium-polar fraction (iii), and elution of the weak-polar fraction (iv). (B) Total ion chromatograms of cefixime tablets (CFXTs) obtained using 2DμCFs-QTOF-HRMS system. CFs: carbon fibers; ACFs: active carbon fibers; MeOH: methanol; FA: formic acid; QTOF-HRMS: quadrupole time-of-flight high-resolution mass spectrometry.
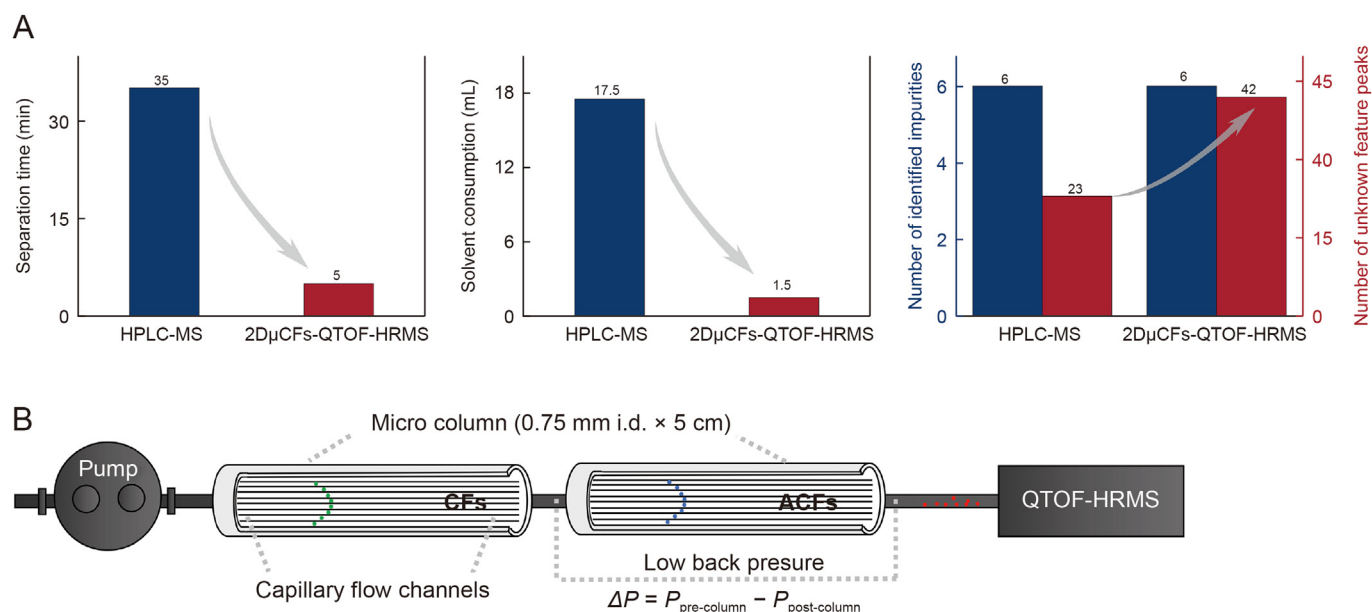
Fig. 2. Advantages of two-dimensional microscale carbon fiber/active carbon fiber system combined with quadrupole time-of-flight high-resolution mass spectrometry (2DμCFs-QTOF-HRMS) compared with high performance liquid chromatography-mass spectrometry (HPLC-MS) for impurity analysis. (A) Technical comparison of the 2DμCFs-QTOF-HRMS system and HPLC-MS for impurity analysis of cefixime tablets (CFXTs). (B) Micro column structure and low-backpressure diagram of the 2DμCFs system. CFs: carbon fibers; ACFs: active carbon fibers; QTOF-HRMS: quadrupole time-of-flight high-resolution mass spectrometry.

than polymerization, contrasting with previous reports, but also has the significant differences in potential activity, absorption, distribution, metabolism, excretion, and toxicity (ADME), and toxicity. The detailed analysis is presented in Supplementary data Part 3 and Tables S4–S6.

2DμCFs-QTOF-HRMS system has excellent analytical capabilities, providing the abundant unknown peaks as the source of potential unknown impurities. Peaks were classified into two classes (>0.1% and <0.1%) based on the ratio of the average mass spectral intensities of the unknown peaks to that of the active pharmaceutical ingredients (APIs). There were 42 unknown peaks for the CFXTs (29, >0.1%; Table S7), 166 for the CFATs (114, >0.1%; Table S8), and 48 for the CPXTs (32, >0.1%; Table S9). The MS information of unknown impurities in CFXTs, CFATs, and CPXTs are compared (Figs. S17–S19). The results indicate that the impurities in CFXTs and CFATs shared several MS peaks and similar relative contents, but the impurities in CFXTs and CPXTs are prone to degradation, whereas those in CFATs exhibited a greater polymerization propensity. Likewise, the full composition analysis of CFXTs from different manufacturers shows that the nature and content of impurities varied among them, probably due to differences in production processes, raw materials, and storage methods (Figs. S20 and S21). The detailed explanations of above results are shown in the Supplementary data Part 3.

Compared to impurity analysis of CFXTs using HPLC-MS, the 2DμCFs-QTOF-HRMS method exhibited greater sensitivity in detecting 42 unknown feature peaks than did HPLC-MS, which detected only 23 unknown feature peaks (Fig. 2A). The detailed description about HPLC-MS data is presented in Supplementary data Part 4, Fig. S22, and Tables S10 and S11. The better analysis results from the 2DμCFs-QTOF-HRMS attribute to a higher resolution of QTOF-HRMS and further the fraction separation of 2DμCFs based on polarity difference of sample components to reduce the ion suppression effects in ESI-MS. While HPLC focus on the full separation of single analyte, in the limited separation time, not all

analytes may be eluted, which also leads to the information loss of some analytes.

According to the above analysis, the 2DμCFs-QTOF-HRMS method demonstrated greater potential for rapid (time, 5 vs. 35 min), eco-friendly (solvent consumption, 1.5 vs. 17.5 mL), and high-efficient (peaks, 48 vs. 29) drug impurity analysis (Fig. 2A). These advantages are attributed to the CFs and ACFs microcolumns (Fig. 2B). As the core units of fraction separation, CFs and ACFs can selectively adsorb the weak- and medium-polar compounds owing to significant differences in surface properties, which are designed dexterously to reduce the co-ionization suppression of compounds with a wide polarity difference. Compared to 2DμCFs-QTOF-HRMS, the impurity intensity obtained by direct-QTOF-HRMS was reduced to different degrees, and the overall reduction range was 19.1%–35.0% (Fig. S23). Furthermore, the linear shape of the CFs, instead of compact particle filling, results in capillary flow channels and efficient fluid transport with high specific permeability and low backpressure [4]. Ordered and miniature CF-filled columns also allow adsorption/desorption processes in a very short time [5], further promoting shorter analysis times, faster separations, and lower organic solvents consumption. It is worth mentioning that CF packing columns are stable over a wide pH range and are compatible with a broad range of solvents, making the 2DμCFs-QTOF-HRMS system more suitable for complex sample analysis.

2DμCFs-QTOF-HRMS shows promise as an alternative to conventional direct-MS and LC-MS for drug impurities screening. It combines direct-MS efficiency with LC-MS advantages, allowing solvent and time-saving procedures, preventing ion suppression. The findings by this study suggest that the content and type of impurities are associated with the generation and brand of oral cephalosporins. Notably, degradation impurities may represent a greater risk than polymerization impurities because of their potentially high content, diversity, and toxicity. Furthermore, owing to the high degree of automation and miniaturization of 2DμCFs technology, it can be combined with

portable MS for the on-site detection and real-time monitoring of drug impurities.

CRediT author statement

Lei Yang: Investigation, Writing - Original draft preparation, Reviewing and Editing; **Yan Wang:** Investigation, Methodology; **Zhao Wang:** Supervision, Methodology; **Meiyu Cui** and **Ruolan Jin:** Investigation, Methodology; **Hai-Bo Shang:** Supervision, Writing - Reviewing and Editing; **Donghao Li:** Resources, Supervision, Writing - Reviewing and Editing, Funding acquisition.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpha.2023.11.007.

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