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Pulse gradient, large-volume injection, high-throughput ultra-performance liquid chromatographic/tandem mass spectrometry bioanalysis for measurement of plasma amrubicin and its metabolite amrubicinol

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ABSTRACT

In this communication, we report the development of a new ultra-performance liquid chromatographic/tandem mass spectrometry (UPLC–MS–MS) assay for measurement of amrubicin (an anthracycline anti-cancer agent) and its active metabolite, amrubicinol, in plasma. The enhanced electrospray ionization signal intensity of the analytes achieved by modifying the mobile phase with formic acid was associated with improvement in the lower limit of quantification. These favorable effects were electrolyte concentration-dependent. In order to maximize assay throughput, we used methanol protein precipitation to prepare the plasma samples, and simplified sample preparation by injecting 40 μL of the supernatant containing methanol at 87.5% (v/v) directly onto the UPLC column without any intermediary solvent evaporation step. The large-volume injection of highly organic supernatant sample increased matrix and elutropic effects, but these drawbacks were respectively overcome by using a 5 mM formic acid-modified mobile phase and a new pulse gradient method. To our knowledge, this is the first report successfully using large-volume injection of strong organic samples with UPLC–MS–MS bioanalysis. The pulse gradient elution also resulted in band compression and enhanced the robustness of the chromatography. The promising new approach illustrated herein is extremely straightforward to optimize, and may be used for UPLC–MS–MS bioanalytical assay of other compounds.

1. Introduction

Liquid chromatography/tandem mass spectrometry (LC–MS–MS) has become the preferred platform for the fast measurement of drugs and their metabolites in biological samples for drug metabolism and pharmacokinetic studies [1–3]. Most applications utilize pneumatically assisted electrospray ionization (ESI) sources to interface the liquid chromatography and mass spectrometry, as opposed to atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI) sources. The reason for this trend is that ESI is better suited for polar, non-volatile, high molecular mass and/or thermally labile analytes. However, current ESI-based platforms are far from being problem-free turnkey solutions. One major problem is that matrix effects resulting from co-eluting residual matrix components of complex biological samples often occur, leading to unacceptable errors and analytic failures [4–6]. The adverse effects have become a major barrier blocking faster development of the higher-throughput bioanalytical methods required for drug discovery/development [7,8].

In our earlier studies [9], we found that inclusion of a very low concentration of $HCOONH_4$ (0.01‰, w/v) in the LC mobile phases substantially enhanced signal intensity and reduced matrix effects. These favorable effects, designated as 'LC-electrolyte effects,' can be achieved in either positive or negative ion ESI modes [9–11], but are not found for APCI [10]. The magnitude of the LC-electrolyte effect on the analyte response depends on the concentration and identity of the electrolyte modifier added into the mobile phase, and is analyte-dependent. Notably, in addition to reducing matrix effects, modifying the LC mobile phase with $HCOONH_4$ also extends

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the upper limit of ESI-based quantification [12]. These observations suggest that the LC-electrolyte effects are associated with increased ESI efficiency and capacity [12].

Despite the high selectivity of MS–MS detection, LC still plays a key role in quantitative bioanalyses. The chromatography step is often optimized with an emphasis on improving the analytical sensitivity by concentrating the analyte on the LC column and by separating analytes from polar matrix components. For these reasons, we proposed the so-called 'trapezoidal-pulse gradient method' for conventional HPLC-based MS–MS analyses of biological samples [10–12].

Researchers have investigated a number of strategies for faster LC without sacrificing efficiency, including the use of monolithic supports, high-temperature LC (up to 90 °C), and ultra-performance LC (UPLC) [13]. UPLC retains the practicality and principles of classical HPLC, but increases the speed, resolution and sensitivity of the procedure by using columns packed with unusually small particles (1.7 μm) and high pressures (up to 1000 bar) [14–16]. Because ultra high-pressure columns require an extremely narrow sample plug to reduce peak spreading, UPLC is usually characterized by small-volume injection and fast injection time.

In context of sample clean-up, many bioanalytical assays for high-throughput measurement use generic and simple protein precipitation methods; however, such methods tend to include more matrix components than other sample preparation techniques [17–19]. Recently, Schuhmacher et al. proposed simple stepwise dilution (10–50-fold) of the supernatant of protein-precipitated plasma samples with the mobile phase to overcome adverse matrix effects for ESI-based detection [8]. Alternatively, Xu et al. reported that increasing the precipitant-to-plasma volume ratio of the protein precipitation-based sample clean-up from 3:1 (v/v) to 6:1 (v/v) significantly reduced co-elution isobaric interference and improved the lower limit of detection [20]. However, these dilution methods are not always possible for sensitivity reasons. In addition, sample elutropic effects due to the high percentage of organic precipitant may reduce chromatographic performance, with large-volume injections of supernatant; especially for high-throughput bioassays based on UPLC.

Amrubicin (ABC) is a potent inhibitor of topoisomerase II [21]. The metabolite amrubicinol (ABN) is 10–100 times more cytotoxic than ABC [22]. Previously, an HPLC-fluorescence-based method was reported for the determination of ABC and ABN in plasma [23], but the method is time-consuming and lacks sensitivity. Here, we describe a new UPLC–MS–MS-based method for simultaneous determination of ABC and ABN from plasma. Notably, we developed a UPLC pulse gradient assay for the direct analysis of supernatant, prepared by methanolic precipitation of the protein components of plasma samples. The method allows use of large-volume injections of highly organic supernatant (40 μL) and allows high sample throughput whilst yielding good analytical sensitivity.

2. Materials and methods

2.1. Reference standards and other materials

Analytical reference standards of amrubicin (ABC; hydrochloride form; 98%) and amrubicinol (ABN; hydrochloride form; 98%) were supplied by Jiangsu Hengrui Medicine Co. (Lianyungang, China). Doxorubicin (hydrochloride form; ≥99%), which was used as an internal standard (I.S.), was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Fig. 1 shows the chemical structures of ABC, ABN, and the I.S. HPLC-grade acetonitrile (CH_3CN; 99.9%) was purchased from Merck (Darmstadt, Germany). Other chemical reagents of analytical grade or better were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). HPLC-grade water was prepared with a Direct-Q 3 UV water purifying system (Millipore, Bedford, MA, USA).

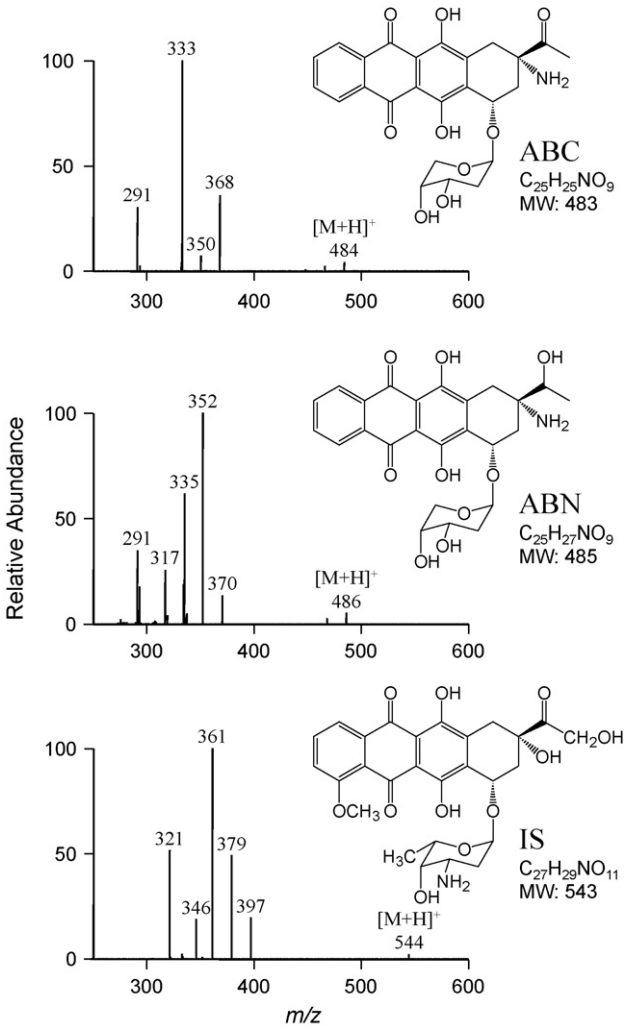

Fig. 1. MS/MS product ion spectra of the protonated molecules ($[M+H]^+$) of amrubicin (ABC; optimal collision energy, 26 V), amrubicinol (ABN; 21 V), and doxorubicin (I.S.; 36 V), as well as their chemical structures.

2.2. Preparation of plasma samples

Plasma samples were prepared using a protein precipitation method at a precipitant-to-plasma volume ratio of 6:1 (v/v). In brief, a 10 μL aliquot of thawed plasma sample was mixed with 60 μL of CH_3OH containing the I.S. (60 ng/mL). The mixture was vortexed for 5 min, and then centrifuged at 15 000 rpm (21 885 × *g*) for 10 min. Forty microliters of the resulting supernatant were directly applied for UPLC–MS–MS analysis.

2.3. UPLC–MS–MS

The UPLC–MS–MS system was assembled from a Waters Acquity UPLC separation module (consisting of a binary solvent manager, a sampler manager equipped with a 50-μL sample loop, a sample organizer, and a column manager; Milford, MA, USA) and a Thermo Fisher TSQ Quantum triple stage quadrupole mass spectrometer (San Jose, CA, USA) with an ESI interface.