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A high throughput approach for simultaneous estimation of multiple synthetic trioxane derivatives using sample pooling for pharmacokinetic studies

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Abstract

The present study describes the application of concept of sample pooling to increase the throughput of pharmacokinetic screening at drug discovery and development stage. An HPLC-UV method for the simultaneous estimation of three synthetic antimalarial compounds 99/357, 99/408 and 99/411 has been developed and validated in rat serum with internal standard for pharmacokinetic profiling. Drug compounds in serum were extracted by two-step liquid–liquid extraction with 2% isopropyl alcohol in *n*-hexane and quantitated using a validated gradient HPLC-UV method, which was made feasible for all compounds using gradient elution scheme. The method was validated in terms of HPLC reproducibility, linearity, specificity, recovery, accuracy and precision, freeze thaw stability and long-term storage stability. Excellent linear relationships ($r > 0.99$) were obtained for calibration as well as analytical standards over a concentration range of 25–1000 ng/ml for three analytes. Recoveries were fond to be >85% for 99/408 and 99/357 and >70% for 99/411. The method developed for three analytes was found to be accurate and precise as bias and percent relative standard deviation (% R.S.D.) values were within limits (<20%). By employing sample pooling approach, plasma level – time profile following single intravenous dose of all three compounds were obtained in a fraction of the time required by conventional single compound dosing and analysis.

Keywords: Sample pooling; Reversed phase liquid chromatography; Pharmacokinetics; Serum; Antimalarial

1. Introduction

The synthesis of large number of compound with the advent of robotics and combinatorial chemistry and HTS results in rapid identification of leads, which requires further preclinical studies before entering to developmental stages. Good pharmacokinetic and metabolism properties are fundamental to the success of the drug candidate, and it is important to have supporting preclinical data before entering to the clinical trials. The success rate of the candidate molecules relies on desired attributes of bioavailability, chemical tractability selectivity and potency. Effective analysis method for generation of the rapid pharmacokinetic data prevents unsuitable candidates reaching later stages and thus maximizing the cost effective utilization of resources. High throughput pharmacokinetic (HTPK) screening approaches provides the rapid and effective ways to generate pharmacokinetic (PK) information and thus economize the time and resources required for the drug discovery. It is therefore necessary to bring the 'traditional' low throughput activities of drug metabolism and pharmacokinetics into the higher throughput arena [1,2]. This leads to birth of novel concept for performing PK studies, i.e. sample pooling and cassette dosing. Sample pooling and Cassette (N-in-one) dosing are the techniques for higher throughput screening in drug development to rapidly assess pharmacokinetic and metabolic profiles of large number of compounds [3].

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Cassette dosing involves administration of multiple compounds to a single animal – sampling – analyzing the samples simultaneously for all the compounds. While cassette dosing seems to be an efficient way to simultaneously screen many compounds, the potential for drug–drug interactions is a problem even at lower doses. Alternatively the sample pooling approach involves administration of one compound per animal, followed by sampling. The samples of same time point for all compounds are pooled and subjected to simultaneous bioanalysis. This one-in-one in vivo approach to accelerate the acquisition of the concentration time data required for pharmacokinetic screening is devoid of potential drug–drug interaction as in N-in-one in vivo pharmacokinetics [4–6].

Both sample pooling and cassette dosing capitalize heavily on tandem liquid chromatography/mass spectrometry due to its high selectivity and sensitivity. In the present study traditional HPLC-UV method is used for simultaneous estimation of multiple synthetic compounds owing to limited availability of LC MS/MS. The size of the sample pool that could be employed under the sample pooling concept depends entirely on the selectivity, specificity and sensitivity of the bioassay developed. In the present method the applicability of conventional bioanalytical techniques like HPLC-UV to sample pooling concept is described using three novel 1,3,4-trioxane antimalarials of artemisinin class [7–12], 99/408, 99/411 and 99/357, developed at Central Drug Research Institute (CDRI), Lucknow.

The present method differs from previously reported HPLC-UV method for 99/357 [13] as it includes two more pharmacologically active trioxane congener and will be applied in estimation of all three compound by novel sample pooling concept, thus giving higher throughput to preliminary pharmacokinetic screening. The assay method reported here was fully validated as per ICH guidelines so as to facilitate reliable and accurate pharmacokinetic profiling. The method was applied to generate intravenous pharmacokinetic profiles of these candidate molecules in male *Sprague-Dawley* rats to illustrate the applicability of sample pooling concept.

2. Materials and methods

2.1. Chemical, reagents and apparatus

The structure of three compounds 99/357, 99/411 and 99/408 (synthetic trioxane derivative) and internal standard is shown in Fig. 1. These compounds (purity >99%) along with internal standard were synthesized by Dr. Chandan Singh in house at the Medicinal Chemistry Division of Central Drug Research Institute (CDRI), Lucknow, India. HPLC grade acetonitrile was obtained from J.T. Baker (USA). *n*-Hexane was procured from J.T. Baker (Philipsburg, USA). HPLC grade isopropyl alcohol (IPA) and dimethyl formamide (DMF) were obtained from Spectrochem Pvt. Ltd. (Mumbai, India). Deionized water (DW) was obtained from Milli Q

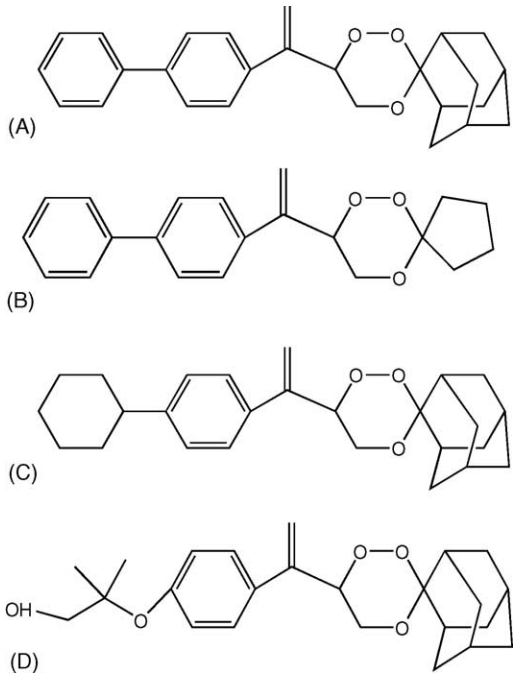

Fig. 1. Chemical structures of (A) 99/357, (B) 99/408, (C) 99/411 and (D) internal standard.

PLUS system (18.2 MΩ cm). All other chemicals were of analytical grade and procured from local sources unless specified.

The HPLC (Shimadzu, Japan) system consisted of a system controller (SCL 10Avp), pump (LC-10Atvp) along with quaternary flow control valve system (FCV-10ALvp) and a degasser (DGU-14A) to pump the mobile phase. The detection was performed using a two-channel UV/vis detector (SPD-10Avp) set at wavelengths 247 and 266 nm. The samples were injected through a syringe loading injector (Model 7725i, Rheodyne, USA) with a fixed 100 μl loop. Chromatographic separations were performed on Spheri-5, RP-18 column, Applied Biosystems, Inc. (100/4.6 mm i.d., 5 mm), coupled with a guard column packed with the same material (30/4.6 mm i.d., 5 mm). The mobile phase was composed of acetonitrile and TDW at a flow rate of 1.5 ml/min. with initial condition of 70% acetonitrile. Before use mobile phase was filtered through 0.22 μm filter and degassed for 20 min in sonicator (Bransonic cleaning Co., USA). Data was analyzed using CLASS-VP software (Shimadzu, Japan) running on a Compaq presario PC. The HPLC system was equilibrated for approximately 30 min at a flow rate of 1.5 ml/min on initial gradient condition before the commencement of the analysis. A vortex-mixer (Thermolyne, USA), Model SVC-220H speed vac concentrator (Savant, NY, USA) and Model K130 centrifuge (BHG Hermle) were used for sample preparation. Serum samples were stored at −60 °C in Ultra Freeze U41085, Ultra Low Freezer (New Brunswick Scientific, USA). Blank serum was obtained from drug free male rats (Sprague-Dawley), which were procured from