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Chemometric resolution of coeluting peaks of eleven antihypertensives from multiple classes in high performance liquid chromatography: A comprehensive research in human serum, health product and Chinese patent medicine samples

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ABSTRACT

A novel chemometric-assisted high performance liquid chromatography method coupled with diode array detector (HPLC-DAD) was presented for the simultaneous determination of eleven antihypertensives from multiple classes in most concerned matrix systems. With the aid of second-order calibration which enables specific information of analytes to be well extracted, the heavily overlapping profiles between analytes and the coeluting interferences can be successfully separated and thus accurately quantified. A great advantage of the novel strategy lies in the fact that the analysis could be carried out with the same isocratic mobile phase (methanol/KH_2PO_4: 58:42, v/v, pH 2.60) in a short time regardless of the changes of matrices, such as human serum, health product and Chinese patent medicine. Both qualitative and quantitative results indicate that the hybrid strategy that using HPLC-DAD coupled with second-order chemometric method would be a high performance approach for the purpose of simultaneously quantifying multiple classes of antihypertensives in complex systems. Additionally, the analytical strategy can potentially benefit drug monitoring in both therapeutic research and pharmaceutical quality control. Moreover, the accuracy and reliability of the proposed methodology has been evaluated using several statistical parameters such as root mean squared error of prediction (RMSEP), figures of merit (FOM) and reproducibility of inter-day analysis.

1. Introduction

High blood pressure, widely called hypertension, is a cardiac chronic disease with a symptom of sustaining rise in systemic arterial blood pressure. It is evident that the elevated blood pressure plays a central role in the pathogenesis of both coronary heart disease and stroke which are the main contributors to the global burden of disease [1–9]. Therefore, the adequate control of hypertension is one of the biggest challenges facing the public health worldwide. The pharmacological treatment options for hypertension can be mainly divided into two categories depending on the severity of the symptoms: monotherapy and combination therapy [6]. In principle, the joint of complementary medicines can not only help to meet the target blood pressure, but also decrease the morbidity and mortality of complications. In practice, many doctors as well as the World Health Organization have been beginning to appreciate the positive outcome and feedback from combining the available antihypertensives [2–7].

Generally, the commonly prescribed western antihypertensive drugs involve diuretics, angiotensin-converting enzyme inhibitors, beta blockers, angiotensin II antagonists, calcium antagonists, alpha blockers, etc. [2,6]. Western medicine treatment is widely adopted due to its rapid onset and high efficiency. Along with the benefits of medication, several risky side effects, such as abuse, unforeseen long term complications, and interaction mechanisms of combining taken drugs, cannot be ignored. More recently, Chinese herbal medicines made from natural herbs have seen an increasing usage as complementary or alternative therapies due to their little side effects. The popularity usages of Chinese herbal medicines demand an urgent necessity for their safety assessment, especially when adulterations in Chinese herbal medicines have been repeatedly reported in various occasions. Therefore, a comprehensive research of co-administrated antihypertensives, in particular, quantification of these compounds in both biological matrix and pharmaceutical preparations is a work of great significance.

Up to now, routine analyses of antihypertensive have been frequently performed by high performance liquid chromatography (HPLC) coupled with different detectors, for instance, ultraviolet

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detector (UV) [10–13], fluorescence detector (FD) [14,15], amperometric detector (AD) [16], diode array detector (DAD) [17–19] and mass spectrometry (MS) [20–22]. However, most works have been focused on quantifying a small amount or one kind of antihypertensives in biological matrix or Chinese herbal matrix. To the best of our knowledge, little work has been published for the analysis of multiple antihypertensives from various classes in different concerned analytical systems involving biological matrix and pharmaceutical preparations. Besides, most of the developed methods were built upon complicated chromatographic conditions. Usually, internal standard, gradient elution and/or long time chromatographic runs were required.

Although tedious pretreatments have been performed before the HPLC analysis, chromatographic separation with acceptable resolutions could not always be achieved owing to either structural similarity among the analytes and coeluting interferences from matrices or the limited time range of per chromatographic run. Since it is essential for the analysis of multiple compounds in complex systems with proper resolution, many efforts have been devoted to enhance the separation capability of chromatographic-based techniques, which is, of course, a troublesome task owning to the lack of generality. Fortunately, with the aid of second-order calibration which makes full use of the information collected in multi-way data array, the separation ability of routine chromatographic-based techniques can be further enhanced by employing 'mathematical separation' to partially substitute the 'physical or chemical separation'. Recently, the strategy that employs hyphenated instruments coupled with second-order calibration algorithms has been successfully applied in a wide range of fields [23–31].

In the present work, eleven antihypertensives were initially assayed using HPLC-DAD coupled with second-order chemometric method based on alternating trilinear decomposition (ATLD) algorithm. The investigated antihypertensives were Triamterene, Indapamide, Propranolol, Furosemide, Carvedilol, Bisoprolol, Doxazosin, Reserpine, Amlodipine, Captopril and Losartan. Compared with other published methods, this proposed strategy manifests several merits: first of all, the combination of chemometric method with HPLC-DAD strategy is originally applied to comprehensively quantify multiple classes of antihypertensives in most concerned biological and Chinese herbs matrix systems (i.e. human serum, health product and Chinese patent medicine samples). Secondly, the introduction of chemometric method, e.g. second-order calibration, enables the separation of analytes in different matrices to be carried out with the same simple isocratic mode and the analysis procedure to be significantly improved. Thirdly, the widely used multiple antihypertensives originating from different complicated analytical systems can be simultaneously analyzed using the same isocratic chromatographic condition, which would be convenient for the purpose of clinical and toxicological monitoring as well as routine pharmaceutical quality control. In addition, the work shown in this paper can be expected as a valuable example of the rapid analysis of multiple antihypertensives in different matrices. The proposed method was evaluated in terms of root mean squared error of prediction (RMSEP), figures of merit (FOM) and reproducibility of inter-day analysis.

2. Experiment

2.1. Chemicals and solutions

Triamterene (TRI, 99%), Indapamide (IND, 99%), Propranolol (PRO, 99%), Carvedilol (CAR, 99%), Bisoprolol (BIS, 99%), Doxazosin (DOX, 99%), Reserpine (RES, 99%), Amlodipine (AML, 99%), Captopril (CAP, 99%), Losartan (LOS, 99%), were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Changsha, China); Furosemide (FUR, 98%) was provided by Adamas Reagent Co., Ltd. (Beijing, China) and all chemicals were used as received. The structure formulas of studied eleven antihypertensives were shown in Fig. 1. Human serum was obtained from Yuanhengjinma Bio-technology Development Co., Ltd. (Beijing, China) and stored at −20 °C in the refrigerator. Luobuma (*Apocynum venetum* leaf, AV) tea and Du-zhong (*Eucommia ulmoides* Oliv., EU) Pingya tablet were purchased from a local pharmacy and stored at room temperature. Methyl alcohol (TEDIA Company, USA) was HPLC-grade, potassium dihydrogen phosphate and concentrated hydrochloric acid were analytical-grade.

All stock solutions (around 1 mg mL^{-1}) were prepared by dissolving the corresponding standards in methanol, stored in brown volumetric flask at −20 °C and were stable at least 3 months (except RES which was freshly prepared).

2.2. Apparatus and chromatographic condition

A 3K30 ultracentrifuge with cooling system (Sigma, USA), a Milli-Q water purification system (Millipore, USA) and a grinder (Zhejiang Yiligongmao, China) were used.

The separation was performed on a LC-20AT liquid chromatographic system (Shimadzu, Japan) coupled with a WondaSil C18 column (5 μm, 150 mm × 4.6 mm, GL Sciences Inc., Japan), a manual injector with a 10 μL loop and a diode array detector. Spectra data were recorded at the range of 190–450 nm. The mobile phase was isocratic and consisted of methanol and 10 mM KH_2PO_4 (pH 2.60) at the ratio of 58:42 (v/v). The flow rate was maintained at 1.0 mL min^{-1} and the column temperature was set to be 30 °C. In all cases, recorded datasets were analyzed in Matlab version 6.5 (Mathworks, Inc.) environment and the programs were developed in the laboratory on a Dell computer (Intel(R) Core2, Dell China) using windows XP (Microsoft) software.

2.3. Sample pretreatment

2.3.1. Human serum samples

Before usage, the drug free human serum was thawed at 36 °C in water bath. The treatment of serum can be summarized as follows: firstly, 2 mL human serum was transferred into a 10 mL centrifugal tube, and then 6 mL of methanol was added and the mixture was sonicated for 30 min [14]. Secondly, the tube was centrifuged for 15 min at 6000 rpm, the supernatant was transferred into another tube. The residue was added another 6 mL methanol, mixed, sonicated, centrifuged and separated in the same way. Thirdly, the extracts were mixture and then evaporated to dryness under nitrogen at 40 °C in water bath. Finally, the residue was reconstituted, adjusted pH to 2.60 (using 2 M HCl) and diluted to 2 mL with mobile phase. An aliquot of 10 μL resulting solution was injected into the HPLC system.

2.3.2. AV samples

AV tea was first smashed, an amount of 5.00 g sample was transferred into a 50 mL centrifuge, 30 mL of methanol was added and then the mixture was treated in the same way as human serum samples described above. At last, the residue was redissolved with 5 mL mobile phase.

2.3.3. EU samples

The treatment of EU was the same as AV samples described above.