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Short communication

UHPLC–MS/MS method with sample dilution to test therapeutic adherence through quantification of ten antihypertensive drugs in urine samples

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ABSTRACT

Nowadays, hypertension represents an important health problem, particularly in developed countries. In some cases the standard therapeutic approaches are not able to reestablish the normal blood pressure values: this condition is called "resistant hypertension". However, a fraction of cases of resistant hypertension are actually due to poor adherence to the prescribed therapy. Therapeutic Drug Monitoring could represent a direct and useful tool to correctly identify non-compliant patients. Nevertheless, high throughput methods for the simultaneous monitoring of a wide panel of drugs in the same analysis are lacking and, furthermore, there is not a generally acknowledged "standard" matrix for this test (plasma or urine).

In this work, we validated a UHPLC–MS/MS assay to quantify ten among the most used antihypertensive agents in urine samples, covering all the current classes: amlodipine, atenolol, clonidine, chlortalidone, doxazosin, hydrochlorothiazide, nifedipine, olmesartan, ramipril and telmisartan.

Both standards and quality controls were prepared in urine matrix. Only 100 μL of each sample were added with 40 μL of internal standard and 860 μL of water:acetonitrile 90:10, acidified with 0.05% formic acid. Chromatographic separation was performed on an Acquity® UPLC HSS T3 1.8 μm 2.1 × 150 mm column, with a gradient of water and acetonitrile, both added with 0.05% formic acid.

Accuracy, intra-day and inter-day precision fitted FDA guidelines for all analytes, while matrix effects resulted reproducible among different urine lots. Method performances were tested on urine samples from hypertensive patients with good results. This simple analytical method could represent a useful tool for the management of antihypertensive therapy.

1. Introduction

Nowadays, the management of hypertensions is becoming increasingly critical for public health systems, even due to the progressive aging of global population. Hypertensive patients are currently treated through combinations of several classes of drugs: diuretics, α/β blockers, calcium antagonists, ACE-inhibitors and sartans [1]. Despite these drugs are able to reestablish normal blood pressure in the vast majority of patients, in a fraction of cases the addition of one or more drugs is mandatory (about 3–30% of treated patients) [2]: this condition is reported as resistant hypertension (RH) [3,4]. The prevalence of RH remains still controversial, mainly because of the difficulty to identify cases of "pseudo-resistance" [5,6]. The latter can be due to clinician-related factors (such as therapeutic inertia, inadequate dose/administration profile or unrecommended combinations) or, more frequently, to patients-related factors, among which poor adherence is one of the

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Table 1
Summary of drug concentrations in standards and quality control samples for each drug.

	STD 9	QC H	QC M	QC L	STD 1 (LLOQ)	LOD
HCTZ (ng/mL)	40000	32000	4000	800	156.25	39.06
TEL (ng/mL)	40000	32000	4000	800	156.25	39.06
OLM (ng/mL)	20000	16000	2000	400	78.12	19.53
ATE (ng/mL)	20000	16000	2000	400	78.12	19.53
CHL (ng/mL)	20000	16000	2000	400	78.12	39.06
NFD (ng/mL)	20000	16000	2000	400	78.12	19.53
DOX (ng/mL)	2000	1600	200	40	7.81	1.95
AML (ng/mL)	2000	1600	200	40	7.81	1.95
CLN (ng/mL)	1000	800	100	20	3.90	1.95
RAM (ng/mL)	200	160	20	4	0.78	0.39

most important [6]. In order to monitor adherence, several indirect methods are reported in literature but their major bias is that they are based on patients self-reported statements. One of the few currently available direct methods to check for adherence is the Therapeutic Drug Monitoring (TDM) of drugs concentrations in biological matrixes, which allows to evidence not only cases of poor adherence but also pharmacokinetic interactions or problems in drug absorption, distribution, metabolism and excretion (ADME). Applying TDM of antihypertensive drugs to recognize poor adherence may prevent some unnecessary invasive therapies employed to manage RH (renal denervation or baroreceptor stimulation) [3,7]. Since patients with RH should be treated with combined therapies [3], the adoption of multiplexed methods results almost mandatory.

In our laboratory it has been recently developed and validated, according to FDA guidelines [8], an ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC–MS/MS) detection method for the quantification of ten antihypertensive drugs in human plasma from patients with suspected RH [9]. However it is essential to point out that plasmatic TDM requires several steps like blood sampling (sometimes difficult and always invasive) and test tube centrifugation, which are not always possible in all the ambulatories. Conversely, the urinary TDM could provide similar (mainly qualitative) information on patients adherence in a simpler and convenient way. Several works previously reported methods for the TDM of antihypertensive drugs in urine, but they are often cumbersome or not thoroughly validated, or comprehensive of only few drugs [10–21]. For these reasons, the aim of this work was the validation, following FDA guidelines [8], of an UHPLC–MS/MS detection urinary method, eligible for a clinical routine use, for the quantification of the same ten antihypertensive drugs in urine samples (from RH/pseudo-RH patients): atenolol (ATE, β-blocker), clonidine (CLN, α2-agonist), doxazosin (DOX, α1-antagonist), amlodipine (AML, calcium antagonist), nifedipine (NFD, calcium antagonist), chlortalidone (CHL, diuretic), hydrochlorothiazide (HCTZ, diuretic), ramipril (RAM, ACE-inhibitor), olmesartan and telmisartan (OLM; TEL, sartans/angiotensin-receptor blockers).

2. Materials and methods

2.1. Chemicals

UHPLC grade acetonitrile (ACN) and methanol (MetOH) were purchased from J.T. Baker (Deventer, Holland). UHPLC grade H_2O was produced with Milli-DI system coupled with a Synergy 185 system by Millipore (Milan, Italy). Blank urine was kindly supplied by healthy donors. ATE, CLN, DOX, AML, NFD, CHL, HCTZ, RAM, TEL and 6,7-dimethyl- 2,3-di(2-pyridyl)quinoxaline (QX), the Internal Standard (IS), were purchased from Sigma-Aldrich Corporation (Milan, Italy); OLM was purchased from Sequoia Research Chemicals (Pangbourne, UK). All powders were stored in the dark, at 4 °C or at room temperature according to instructions, in order to prevent any possible degradation.

2.2. Stock solutions, internal standard, standards, and quality controls

Stock solutions (1 mg/mL) were prepared as below: AML, CHL, HCTZ, NFD, OLM, RAM and QX stock solutions in a mixture of H_2O:MetOH 5:95 (v:v); DOX and TEL in Dimethyl sulfoxide (DMSO):MetOH 50:50 (v:v); ATE and CLN in H_2O:MetOH 50:50 (v:v) and pure H_2O, respectively. Stock solutions were stored at −20 °C until use (less than 1 month), with the exception of CLN stock solution which was stored at 4 °C (less than 1 months). Single aliquots of Standard 9 (STD9) and Quality Controls (QCs) were prepared by independently spiking blank urine from healthy donors with stock solutions and then stored at −80 °C. Calibration range and QC levels are summarized in Table 1. IS working solution was prepared by diluting 4 μL of QX stock solution in 4 mL of H_2O:MetOH [50:50] (final concentration 1000 ng/mL) at each analytical session.

2.3. Sample preparation

Because of NFD photodegradation all preliminary steps have to be performed using amber tubes and keeping urine samples far from light sources. Due to high concentration of antihypertensive drugs in urine, sample preparation consists in a simple 1:10 dilution in order to avoid signal saturation and to reduce system contamination and matrix effect: 100 μL of urine samples and 40 μL of IS working solution were added with 860 mL of H_2O:ACN 90:10 (v:v) + 0.05% formic acid in amber PTFE tubes. Samples were vortex-mixed for 10 s and centrifuged at 21000 × *g* at 4 °C for 10 min (with low brake) in order to eliminate any possible solid residue (eg. uric acid crystals). In some cases the formation of a very small pellet can be observed. The supernatant was transferred in bulk vials: 0.3 μL of the resulting extracts were injected into the UHPLC–MS/MS system.

2.4. UPLC–MS/MS instruments and chromatographic conditions

The chromatographic conditions were kept similar to our previously published method for plasma quantification of the same drugs [9] and are resumed as follows: a Shimadzu Nexera X2 UHPLC system coupled with LCMS-8050 tandem mass detector was used for the chromatographic analysis. Chromatographic separation was performed through an Acquity ® UPLC HSS T3 1.8 μm 2.1 × 150 mm (Waters, Milan, Italy), protected by a physical frit [0.2 μm, 2.1 mm] (Waters, Milan, Italy) precolumn, at 40 °C using a column thermostat, with a gradient (Table 2) of two mobile phases: phase A (H_2O + formic acid 0.05%) and phase B (ACN + formic acid 0.05%). The instrument was settled in positive electrospray ionization mode (ESI+) for all drugs, except for HCTZ, which was detected