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Simultaneous determination of L-arginine and 12 molecules participating in its metabolic cycle by gradient RP-HPLC method Application to human urine samples

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ABSTRACT

We have developed and described a highly sensitive, accurate and precise reversed-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous determination of L-arginine and 12 molecules participating in its metabolic cycle in human urine samples. After pre-column derivatization with *ortho*-phthaldialdehyde (OPA) reagent containing 3-mercaptopropionic acid (3MPA), the fluorescent derivatives were separated by a gradient elution and detected by fluorescence measurement at 338 nm (excitation) and 455 nm (emission). L-Arginine (ARG) and its metabolites: L-glutamine (GLN), N^G-hydroxy-L-arginine (NOHA), L-citrulline (CIT), N^G-monomethyl-L-arginine (NMMA), L-homoarginine (HARG), asymmetric N^G,N^G-dimethyl-L-arginine (ADMA), symmetric N^G,$N^{G'}$-dimethyl-L-arginine (SDMA), L-ornithine (ORN), putrescine (PUT), agmatine (AGM), spermidine (SPERMD) and spermine (SPERM) were extracted in a cation-exchange solid-phase extraction (SPE) column and after derivatization separated in a Purospher® STAR RP-18e analytical column. The calibration curves of analysed compounds are linear within the range of concentration: 45–825, 0.2–15, 16–225, 12–285, 0.1–32, 15–235, 0.1–12, 0.1–12, 10–205, 0.02–12, 0.1–24, 0.01–10 and 0.01–8 nmol mL^{-1} for GLN, NOHA, CIT, ARG, NMMA, HARG, ADMA, SDMA, ORN, PUT, AGM, SPERMD and SPERM, respectively. The correlation coefficients are greater than 0.9980. Coefficients of variation are not higher than 6.0% for inter-day precision. The method has been determined or tested for limits of detection and quantification, linearity, precision, accuracy and recovery. All detection parameters of the method demonstrate that it is a reliable and efficient means of the comprehensive determination of ARG and its 12 main metabolites, making this approach suitable for routine clinical applications. The levels of analysed compounds in human urine can be successfully determined using this developed method with no matrix effect.

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1. Introduction

L-Arginine ((2S)-2-amino-5-(diaminomethylideneamino) pentanoic acid) is classified as a semiessential or conditionally essential amino acid, that plays an important function in the metabolism of an organism. Discovery that nitric oxide (NO) is synthesized from L-arginine (ARG) by nitric oxide synthase (NOS) and works diastolic for vessels, has changed the perception of ARG role in many physiological and pathological processes and it had an influence on increased interest in this amino acid role in biochemistry, physiology and nutrition of human and animals. NO is an important signal-transduction molecule that plays a significant role in the regulation of cardiovascular functions [1–14].

Particularly huge interest in researches of ARG metabolic pathways with NO release has come since R. Furghott, L. Ignaro and F. Murad received Nobel Prize for their work on NO part in cardiovascular functions (scientists were awarded on medical researches field in 1998). Simultaneously, ARG participation in diseases treatment and troubles caused by deficiency of NO in organism (e.g. endothelial dysfunction [15], all types of arterial hypertensions [9,16–19], dyslipidemia [15,20], neurotransmission [1,21–23], hyperglycemia [24], renal failure [9,25–28], vascular disease has increased [9,29]). NO role in liver metabolism was widely described by Alexander in review article [30].

ARG is the precursor for the synthesis of proteins and other molecules of great biological importance. Direct substrate for ARG synthesis is L-citrulline (CIT), which is synthesized in small intestine [31]. Stable intermediate synthesized from ARG into NO in transformation reaction is N^G-hydroxy-L-arginine (NOHA) belonging to type I and II arginase inhibitor [32]. L-Glutamine (GLN) is indispensable precursor for proteins and other amino acids like L-glutamate, ARG, ORN, CIT, proline and nucleotides, glucose or amino sugar synthesis [33]. In modification process after translate ARG is methylated by *N*-methyltransferase to N^G-monomethyl-L-arginine (NMMA), asymmetric N^G,N^G-dimethyl-L-arginine (ADMA) and symmetric N^G,$N^{G'}$-dimethyl-L-arginine (SDMA) [34]. As a result of ARG catalytic hydrolysis is synthesized from L-ornithine (ORN) and urea [35]. Agmatine (AGM) and CO_2 are formed in decarboxylation process of ARG, catalysed by arginine decarboxylase (ADC) [36]. Precursors in polyamines (putrescine (PUT), spermidine (SPERMD) and spermine (SPERM)) synthesis, compounds needed for proliferation, differentiation and normal cells work are ARG and ORN. For their synthesis ARG is necessary, which owing to arginase is transformed into ORN. This turn is a substrate for PUT synthesis which is next, transformed to SPERM and SPERMD [3,37].

Many HPLC methods applied in the analysis of ARG and some of its metabolites in different biological fluids and tissues (plasma, serum, saliva, cerebrospinal fluid, cancer cells, rat brain or *Lathyrus sativus*) have been described in the literature. HPLC analysis of ADMA and other methylated ARG analogs in biological fluids was described in review article by Teerlink [38].

Only relatively small amount of applications refers to determination of the above-mentioned compounds in urine. ARG, monomethylated (NMMA) and dimethylated (ADMA and SDMA) arginines, and HARG in human urine samples were described [39–44]. GLN in urine samples was determined frequently simultaneously with ARG and some of its metabolites (e.g. CIT, ADMA, SDMA and ORN) [42,45–47]. Several chromatographic methods have been published for quantification of PUT, SPERMD and SPERM in urine [48–55]. Review articles about metabolism and determination of selected polyamines (e.g. PUT, SPERMD, and SPERM) were written [56,57].

Generally applied reagent for ARG derivatization, some of its metabolites and selected polyamines (PUT, SPERMD and SPERM) in human urine samples were OPA [38–40,42,45–47,53,58,59]. Review articles about other reagents used for derivatization of different amino acids and various polyamines were presented by Teerlink [38], Molnár-Perl [60] and Fekkes [61].

Besides fluorescence detection for determination some of above-mentioned compounds after separation by HPLC or GC methods UV–vis spectrophotometric [62–65], MS [41,43,49,52,66–71] or electrochemical [72–76] detection was applied by authors.

Though ARG and its metabolites are well known to affect various cardiovascular physiologies, the currently available literature is still not sufficient to validate the prophylactic/therapeutic efficacy of ARG. Therefore it is necessary to develop one chromatographic system which could provide simultaneous determination of ARG and its metabolites.

The purpose of this study was to develop a simple and accurate analytical method to determine ARG and its 12 metabolites (GLN, NOHA, CIT, NMMA, HARG, ADMA, SDMA, ORN, PUT, AGM, SPERMD and SPERM) based on pre-column derivatization, reversed-phase, gradient elution and fluorescence detection in human urine samples. It is necessary to underline the fact that it is the first elaborated and validated method which enables simultaneous determination of ARG and 12 molecules participating in its metabolic cycle in 1 chromatographic system in human urine samples. To our knowledge, this is the first analytical method that allows the simultaneous determination of ARG and its 12 primary metabolites in human urine.

2. Experimental

2.1. Materials

L-Glutamine (≥99% purity), L-arginine (≥98.5% purity), L-ornithine monohydrochloride (~99% purity) and asymmetric N^G,N^G-dimethyl-L-arginine dihydrochloride were obtained from Sigma–Aldrich (Schnelldorf, Germany). L-Citrulline (≥99% purity), N^G-monomethyl-L-arginine acetate salt (≥95% purity), L-homoarginine hydrochloride (≥98% purity), putrescine (≥99% purity), agmatine sulfate salt (≥99% purity), spermidine (≥99.5% purity), spermine (≥99% purity), 3-mercaptopropionic acid (≥99% purity) and *ortho*-phthaldialdehyde (≥99% purity) were purchased from Fluka BioChemika (Darmstadt, Germany). N^G-Hydroxy-L-arginine monoacetate salt (>98% purity) and symmetric N^G,$N^{G'}$-dimethyl-L-arginine dihydrochloride were kindly provided by Calbiochem (Schnelldorf, Germany). The molecular structures