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# Determination of doxazosin and verapamil in human serum by fast LC–MS/MS: Application to document non-compliance of patients

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ABSTRACT

A rapid and sensitive method using liquid chromatography–tandem mass spectrometry (LC–MS/MS) for simultaneous determination of doxazosin and verapamil in human serum has been developed. Trimipramine-$d_3$ as an isotopic labelled internal standard was used for quantification. Serum samples were prepared by simple liquid–liquid extraction with mixture of *tert* butyl methyl ether and ethyl acetate (1:1, v:v). The analytes and internal standard were separated on C18 column using an isocratic elution with 5 mM ammonium formate with 0.02% formic acid and 0.02% formic acid in acetonitrile (55:45, v:v) at a flow rate of 1.1 mL/min. Positive TurboIonSpray mass spectrometry was used with multiple reaction monitoring of the transitions at: $m/z$ 455.3 → 165.2 and 150.2 for verapamil, $m/z$ 452.2 → 344.4 and 247.4 for doxazosin, $m/z$ 298.2 → 103.1 for trimipramine-$d_3$. Linearity was achieved between 1 and 500 ng/mL ($R^2 \geq 0.997$) for both analytes. An extensive pre-study method validation was carried out in accordance with FDA guidelines. This assay was successfully applied to determine the serum concentrations of doxazosin and verapamil in suspect non-compliance patients.



## 1. Introduction

Essential or primary hypertension refers to high blood pressure with no identifiable cause and remains a major modifiable risk factor for cardiovascular disease. Essential hypertension is nowadays one of the most common disorders of western civilisation. The estimates predict further increase in number of adults suffering from hypertension by about 60% till 2025, when 1.56 billion (1.54–1.58 billion) of people are predicted to have hypertension [1]. If lifestyle and diet modifications are not satisfactory, medical treatment with antihypertensive drugs is necessary. Resistant hypertension is defined as a state, when the treatment with at least 3 antihypertensive drugs (including a thiazide diuretic) is not able to normalize the blood pressure. This is a relatively frequent, but often omitted problem. Exclusion of secondary hypertension, including endocrine hypertension (mainly primary aldosteronism, but also pheochromocytoma and hypercortisolism) is recommended in these patients. Therefore the patients are switched to a drug combination of alpha 1 adrenoreceptor antagonists (A1AAs) [2,3] and/or calcium channel blockers (CCBs) [4–6], which does not affect plasma concentration of the endogenous hormones connected to secondary hypertension. Doxazosin and verapamil (Fig. 1) are used as the substitutive antihypertensive drug combination [7–10].

Doxazosin mesylate [(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(1,4-benzodioxan-2-yl-carbonyl) piperazine monomethansulphonate], a quinazoline derivate, is a long-acting postsynaptic A1AA [11] displaying similar efficacy to that of other A1AAs, beta adrenoreceptor antagonists, diuretics, CCB and angiotensin-converting enzyme inhibitors [12]. Like structurally similar prazosin, doxazosin exerts its antihypertensive effect by reducing total peripheral resistance [13]. Verapamil, (±)-(alpha)[3-[[2-(3,4-di-dimethoxyphenyl)ethyl] methylamino]propyl]-3,4-dimethoxy-(alpha)-(1-methylethyl) monohydrochloride, is a selective CCB effective in the treatment of hypertension, arrhythmia and angina pectoris [10,14,15].

Due to wide use of doxazosin and verapamil, several analytical methods for the determination of verapamil and doxazosin individually have been described in literature. Verapamil was preferably determined in human plasma or in other biological specimens by liquid chromatographic methods coupled with ultra-violet detector (HPLC-UV) [16–20], fluorimetric detection (HPLC-FLR) [21–25] and mass spectrometric detection (LC–MS) [26–28]. Methods

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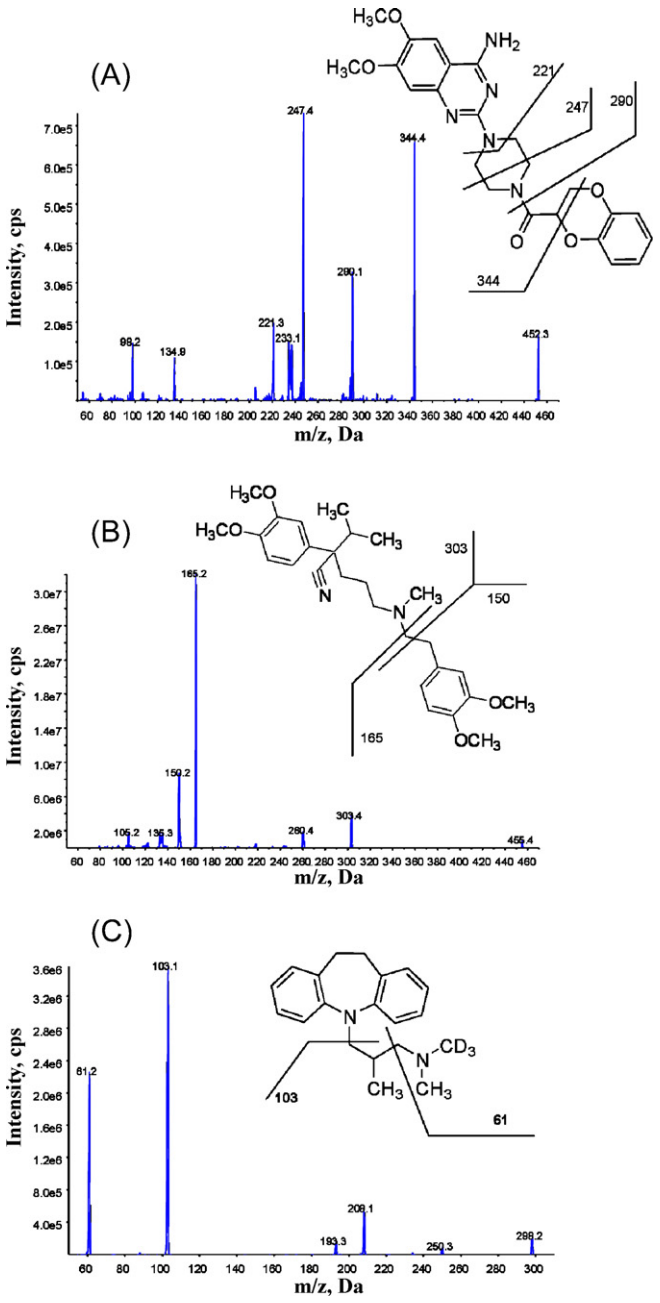


**Fig. 1.** Chemical structures and product ion spectra of $[M+H]^+$ of doxazosin (A), verapamil (B) and trimipramine-$d_3$ (C).

involving gas chromatography (GC) were also published [29–32]. Determination of doxazosin in pharmaceutical formulations and biological matrices was performed using HPLC-UV [33], HPLC-FLR [34–40] and LC–MS [41–43]. Doxazosin was also analysed using electrochemical techniques [44,45]. A method for simultaneous determination of both analytes was not published so far.

Determination of both doxazosin and verapamil in biological matrices is routinely used in pharmacokinetic studies. However, structurally related antihypertensive agents are used as an internal standard (IS), which prevents possible application of such methods into real clinical settings because there is a high risk of interferences by co-medications. Structures like tetrazosin [42], prazosin [34,35,37,39,41], cisapride [43], propranolol [24,36,38], metoprolol [26], dextrometorphan [20] or gallopamil [25] have been used as an IS in methods for applications in controlled pharmacokinetic studies.

We describe a fast, selective and accurate LC–MS/MS method for the simultaneous quantification of verapamil and doxazosin using trimipramine-$d_3$ as an IS in this paper. This method was specially developed to confirm suspect non-compliance of patients treated with verapamil and (or) doxazosin, since non-compliance has been previously reported as a prevalent cause of pseudo-resistant hypertension [46,47]. This method is easy to provide and is applicable for routine determination of both medicaments in clinical practice. The time non-consuming liquid–liquid extraction (LLE) was used for sample pre-treatment. Additionally, we used high resolution mass spectrometry for the interpretation of unknown fragment ion spectra of doxazosin.

## 2. Experimental

### 2.1. Chemicals and reagents

Standard of doxazosin mesylate (99.4%) was kindly supplied by Zentiva (Prague, Czech Republic). Verapamil hydrochloride (99.0%) was purchased from Sigma Aldrich (Steinheim, Germany) and trimipramine-$d_3$ (99.0%) was obtained from Alltech (Prague, Czech Republic). Formic acid (p.a.) and ammonium formate (p.a.) were purchased from Sigma Aldrich (Steinheim, Germany). Acetonitrile (HPLC grade) and extra pure solvents *tert*-butyl methylether (TBME) and ethyl acetate were obtained from Merck (Darmstadt, Germany). Deionised water was produced in-house using a Milli-Q-System from Millipore (Bedford, MA, USA).

### 2.2. Instrumentation

The chromatographic separation was performed on a 1200 RRLC (Agilent, Waldbronn, Germany), consisting of a degasser, binary pump, autosampler and thermostatted column compartment. The MS/MS analysis was performed using a 3200 Q-trap triple quadrupole/linear ion trap mass spectrometer with a TurboIonSpray source (MDS Sciex, Ontario, Canada). For data analysis was used Analyst software version 1.5.1.

### 2.3. LC–MS/MS

Chromatographic separation was achieved with an Agilent Zorbax Eclipse XBD-C18 column (1.8 μm, 50 × 4.6 mm I.D.), protected by a C18 security guard cartridge (4 × 2 mm I.D.). Isocratic elution occurred with (A) 5 mM ammonium formate with 0.02% formic acid and (B) 0.02% formic acid in acetonitrile (55:45, v:v) at a flow rate of 1.1 mL/min. The mobile phase was thermostatted at 40 ± 0.5 °C. The mass spectrometer operated in positive TurboIonSpray mode and selected reaction monitoring (SRM) was used for data acquisition of both analytes and IS. The following transitions were monitored: *m/z* 455.3 → 165.2 and 150.2 for verapamil, *m/z* 344.4 → 165.2 and 247.4 for doxazosin, *m/z* 298.2 → 103.1 for IS. The underlined transitions were used for quantification. The MS parameters for the analysis were as follows: ion source temperature 550 °C; ion-spray voltage 5000 V; nebulizer gas 45 psi; auxiliary gas 50 psi; curtain gas 10 psi and medium collision gas. Conditions of mass spectrometric detection were optimized by direct infusion of standard solutions into the MS. The final parameters settings are shown in Table 1.

### 2.4. High resolution mass spectrometry

High resolution exact mass MS/MS spectra for doxazosin were obtained with an LTQ Orbitrap XL hybrid mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) equipped with an electrospray ion source. The spectrometer was operated in positive