

# UPLC-MS/MS Method for the Analysis of Cardiovascular Medications in Putrefied Specimens obtained from an Exhumation Autopsy

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## Introduction

Cardiovascular diseases are closely related to several risk factors such as high blood pressure, obesity, high blood levels of cholesterol and triglycerides and insulin resistance and are the major cause of death in adults and the elderly in the majority of the developed countries. The risk of cardiovascular disease can be reduced through pharmacological treatment, however to achieve that, most patients require a combined cardiovascular medication involving different drug classes which have different targets and mechanisms of action to regulate each factor separately. In the case of several compounds used in combined cardiovascular therapy, it is very important to monitor the blood concentrations of drugs to understand their pharmacokinetics and pharmacodynamics and also to detect misuse of these drugs to reveal accidental or intentional intoxications. [1-3] Therefore, the development of an assay that can detect a range of cardiovascular medications using a single small sample may be of great importance in the forensic context.

The aim of this work was to develop a versatile analytical method that allows the simultaneous identification of several different substances used for cardiovascular diseases treatment in putrefied specimens collected in the context of an exhumation performed several decades after death. For this purpose an UPLC-MS/MS method was developed for the qualitative analysis of 26 substances that included: anticoagulants; angiotensin converting enzyme inhibitors; angiotensin II receptor blockers; beta-blockers; calcium channel blockers; diuretics; statins; vasodilators and others. During autopsy, in spite of the corpse was partially mummified it was possible to collect body tissues from different anatomic regions, namely: encephalic mass, heart, lungs, liver, stomach, kidney, bladder and colon.

## Experimental

Sample Preparation

Each sample was previously diluted, homogenized and subjected to a sample precipitation procedure according to the following **figure**:

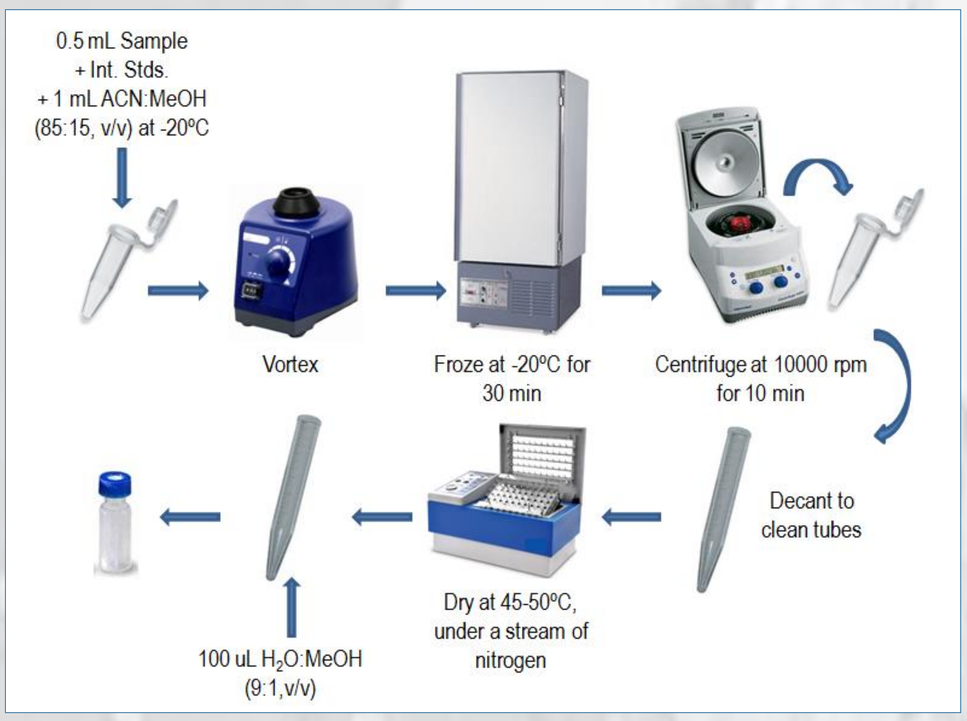


**Figure 1.**Sample preparation procedure

UPLC-MS/MS

- ❑ Equipment: Waters Acquity UPLC/TQ Detector (Triple quadrupole mass spectrometer).
- ❑ Column: Acquity UPLC® HSS T3 (100 mm x 2.1 mm i.d., 1.8 µm).
- ❑ Mobile phase: Solvent A – ACN; Solvent B – Ammonium formate 2 mM (0.1% HCOOH).
- ❑ Flow: 0.3 mL/min.
- ❑ Gradient: 5% (A); 5-65% (A) in 3.5 min; 65-95% (A) in 4.5 min; 95%(A) for 2 min; 95-5% (A) in 0.1 min; 5% (A) for 2.4 min.
- ❑ Ionization/Acquisition mode: ESI +/- ; MRM (see **table 1**)

## Results

Due to the high complexity and uniqueness of the specimens analyzed the method was assessed in terms of the recovery and limits of detection (LOD) using the standard addition method. The results obtained for the LODs and recovery are presented in **table 2.**

**Table 1.** MRM transitions, cone voltage, collision energy and retention time (RT) of the analytes and internal standards (IS).

| Substance | ESI (+/-) | MRM Transitions (m/z) | Cone (V) | Collision (eV) | RT (min) |
|---|---|---|---|---|---|
| Methyldopa | + | 212.0 > 139.1/166.1 | 25 | 16 | 1.15 |
| Ethylephrine | + | 182.0 > 91.2/164.1 | 25 | 22/12 | 1.33 |
| Atenolol | + | 267.0 > 145.2/190.1 | 40 | 26/20 | 1.50 |
| Lisinopril | + | 406.1 > 84.2/246.2 | 45 | 24/20 | 1.83 |
| Clonidine | + | 230.0 > 44.3/160.1 | 40 | 36 | 1.91 |
| Hydrochlorothiazide | - | 296.0 > 205.0/269.0 | 40 | 24/18 | 2.10 |
| Triamterene | + | 254.0 > 104.1/237.1 | 60 | 36/28 | 2.11 |
| Sulfameter (IS) | + | 281.0 > 108.0 | 35 | 22 | 2.52 |
| Propanolol | + | 260.0 > 116.2/183.1 | 40 | 18 | 3.02 |
| Enalapril | + | 377.1 > 234.2/303.1 | 30 | 20 | 3.03 |
| Diltiazem | + | 415.0 > 178.0/370.0 | 40 | 24/16 | 3.30 |
| Digoxin | + | 798.3 > 651.0/781.0 | 25 | 12 | 3.35 |
| Carvedilol | + | 407.0 > 100.2/283.2 | 40 | 28/20 | 3.40 |
| Pravastatin | - | 423.1 > 101.2/321.2 | 40 | 24/16 | 3.40 |
| Furosemide | - | 329.0 > 205.0/285.0 | 40 | 20/14 | 3.48 |
| Ramipril | + | 417.1 > 234.2/343.2 | 30 | 22 | 3.54 |
| Verapamil | + | 455.2 > 165.2/303.0 | 50 | 28 | 3.64 |
| Reserpine | + | 609.3 > 195.2/397.1 | 60 | 34/28 | 3.77 |
| Methyldigoxin | + | 812.3 > 651.0/795.0 | 25 | 12 | 3.89 |
| Losartan | + | 423.0 > 207.1/405.1 | 30 | 12/24 | 3.92 |
| Temilsartan | + | 515.1 > 276.2/497.1 | 45 | 46/38 | 4.05 |
| Nifedipine | + | 347.0 > 271.1/315.0 | 20 | 12/8 | 4.37 |
| Valsartan | + | 436.1 > 235.1/291.1 | 30 | 16 | 4.41 |
| Oleandrin (IS) | + | 577.4 > 373.1 | 30 | 14 | 4.48 |
| Warfarin | + | 309.0 > 163.0/251.0 | 30 | 14/18 | 4.61 |
| Atorvastatin | + | 559.1 > 292.1/440.1 | 40 | 26 | 4.83 |
| Lovastatin | + | 405.1 > 199.2/285.1 | 25 | 12 | 6.32 |
| Simvastatin | + | 419.1 > 199.2/285.2 | 40 | 12 | 6.91 |

**Table 2.** Results obtained for the limit of detection (LOD) for the samples: S1 (encephalic mass); S2 (heart); S3 (lung); S4 (liver); S5 (stomach); S6 (kidney); S7 (bladder) and S8 (colon) and for the recovery.

| | LOD (ng/mL) | | | | | | | | Rec. (%) |
|---|---|---|---|---|---|---|---|---|---|
| Substance | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | |
| Methyldopa | 180 | 70 | 140 | 250 | 280 | 440 | 330 | 500 | 48 |
| Ethylephrine | 5 | 15 | 5 | 50 | 25 | 20 | 40 | 40 | 71 |
| Atenolol | 0.7 | 3.3 | 1.5 | 5 | 5 | 1.5 | 5 | 5 | 74 |
| Lisinopril | 1.8 | 4.3 | 5 | 5 | 5 | 5 | 5 | 5 | 55 |
| Clonidine | 50 | 50 | 50 | >50 | >50 | >50 | >50 | >50 | 82 |
| Hydrochlorothiazide | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 73 |
| Triamterene | 2 | 5 | 5 | 5 | 5 | 5 | 15 | 5 | 73 |
| Propanolol | 1 | 5 | 5 | 20 | 3,6 | 20 | 15 | 5 | 83 |
| Enalapril | 0.8 | 2.2 | 1.8 | 1.8 | 1.4 | 1.8 | 1.5 | 1.4 | 76 |
| Diltiazem | 0.2 | 5 | 5 | 5 | 1.4 | 5 | 15 | 5 | 85 |
| Digoxin | 2.5 | 5 | 5 | 5 | 5 | 5 | 4.1 | 5 | 83 |
| Carvedilol | 2.8 | 15 | 5 | 30 | 5 | 5 | 25 | 0.3 | 83 |
| Pravastatin | 0.6 | 1.2 | 0.6 | 1.5 | 0.2 | 0.5 | 0.9 | 0.3 | 84 |
| Furosemide | 5 | 5 | 2.7 | 5 | 4.4 | 4.2 | 5 | 3 | 80 |
| Ramipril | 0.2 | 1.9 | 1.2 | 1.2 | 1.2 | 1 | 1 | 1.3 | 79 |
| Verapamil | 0.2 | 1 | 1.3 | 5 | 0.9 | 2.2 | 5 | 5 | 83 |
| Reserpine | 1.2 | 5 | 5 | 25 | 2.1 | 5 | 7.4 | 50 | 76 |
| Methyldigoxin | 2.3 | 5 | 5 | 5 | 5 | 5 | 5 | 4 | 81 |
| Losartan | 1.2 | 2 | 1.9 | 3.6 | 0.9 | 1.9 | 0.9 | 1.7 | 85 |
| Temilsartan | 0.4 | 3.1 | 1.4 | 2.8 | 0.8 | 2.7 | 2.8 | 2.6 | 93 |
| Nifedipine | 0.6 | 0.6 | 0.3 | 0.9 | 0.5 | 0.3 | 0.1 | 0.3 | 75 |
| Valsartan | 0.1 | 0.2 | 0.2 | 0.2 | 0.1 | 0.1 | 0.1 | 0.2 | 54 |
| Warfarin | 0.1 | 0.2 | 0.1 | 0.2 | 0.1 | 0.05 | 0.05 | 0.1 | 82 |
| Atorvastatin | 0.5 | 2.4 | 0.5 | 1.8 | 0.6 | 2.2 | 0.6 | 0.4 | 95 |
| Lovastatin | 0.6 | 1 | 0.5 | 0.7 | 1.2 | 2.4 | 1.5 | 4 | 107 |
| Simvastatin | 0.8 | 0.5 | 0.6 | 1.2 | 0.2 | 1.3 | 0.9 | 5 | 79 |

## Conclusions

The analysis of a collection of substances with different physicochemical properties and a wide range of expected concentrations in biological matrices represents a challenge due to the difficulty of developing a proper extraction procedure and an analytical method adequate for all the analytes detection. That challenge is even higher when the biological samples are not conventional matrices such as the putrefied specimens collected from an exhumation autopsy, as presented in this work. The UPLC-MS/MS method developed together with a very simple sample preparation procedure showed adequate levels of performance with recovery range from 48% to 107% and LOD values in the range of 0.05 – 50 ng/mL for all the substances, except for the methyldopa with estimated LODs that ranged from 70 - 500 ng/mL.

## References

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