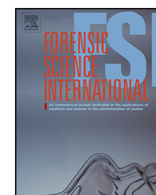
Forensic Science International 267 (2016) 110–114

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint



# Concurrent estimation of metabolite concentrations along with parent drug quantification in post-mortem blood

Jenni Viinamäki [1,*], Ilkka Ojanperä [1,2]

[1] University of Helsinki, Department of Forensic Medicine, P.O. Box 40, FI-00014 University of Helsinki, Finland
[2] National Institute for Health and Welfare, Forensic Toxicology Unit, Helsinki, Finland

ARTICLE INFO

*Article history:*
Received 19 April 2016
Received in revised form 10 August 2016
Accepted 20 August 2016
Available online 28 August 2016

*Keywords:*
Drug metabolite quantification
Universal detection
Corona charged aerosol detection
Photodiode array detection
Ultra-high performance liquid chromatography
Post-mortem blood

ABSTRACT

There is a constant demand for the quantification of drug metabolites within post-mortem toxicology. Especially electrospray ionization–mass spectrometry techniques necessitate that calibration is carried out using primary reference standards due to the non-uniform ionization efficiency between parent drugs and their metabolites. As reference standards for metabolites are not readily available and their costs are high, alternative methods for immediate quantification are required. In this study, ultra-high performance liquid chromatography coupled with photodiode array detection and corona charged aerosol detection was utilized for the concurrent quantification of 23 drug metabolites using the corresponding parent drug for calibration. Based on this secondary calibration, the quantitative results for the *N*-demethylated metabolites by each detector were similar to those obtained by the ordinary calibration using reference standards. For *O*-demethylated metabolites, the differences in detector response caused somewhat larger biases using the secondary calibration. Using the validated secondary calibration, the blood sample data gathered from 633 post-mortem cases was retrospectively re-processed to discover the combined metabolite–parent concentrations and metabolite to parent ratios for six toxicologically relevant drugs. These results, representing all causes of death, were compared to published data from therapeutic drug monitoring and post-mortem toxicology.

© 2016 Elsevier Ireland Ltd. All rights reserved.

## 1. Introduction

Drug metabolite plasma concentrations are informative in therapeutic drug monitoring (TDM), as several drugs have pharmacologically active metabolites. The therapeutic ranges of some antipsychotics and antidepressants are commonly expressed as the combined concentration of the parent drug and its active metabolite [1]. In the guidelines for TDM in psychiatry, the ranges of normal metabolite to parent ratios for several drugs have been established [2]. Even the determination of less active metabolites is recommended in order to ascertain compliance or the patient's capability to metabolize drugs [1,2]. In post-mortem toxicology, however, related data on metabolite blood concentrations of statistical relevance is limited to only few publications [3], while most of the data is scattered in miscellaneous case notes and case series [4]. The lack of data is largely due to the fact that reference standards for many less active metabolites, for example hydroxyl derivatives, are not readily available and their costs are high, which in turn does not motivate the effort required to routinely analyze metabolites in casework. In the absence of reference standards, the two most important obstacles in quantification are the lack of a suitable analytical tool that possesses a uniform response to the parent drug and its metabolite, and the possible difference in the extraction recovery between the parent and metabolite [5].

Liquid chromatography-mass spectrometry (LC-MS) techniques are widely used in post-mortem toxicology due to their selectivity and sensitivity. However, the electrospray ionization (ESI) efficiency can be significantly dependent on analyte structure [6,7]. Furthermore, LC gradients affect the MS response, as compounds with different retention times are sprayed into the mass spectrometer in different solvent compositions [6]. Using nanospray ionization (NSI) [5,6,8,9] or captive spray ionization (CSI)-LC-MS [10,11] reduces differences in the ionization efficiency between drugs and their metabolites, but these techniques are currently not robust enough to be a strong option in post-mortem toxicology.

UV detection has previously been used for the quantification of drug metabolites using the parent drug for calibration [12,13]. However, even with apparently similar UV spectra the quantification can be erroneous if the chemical changes take place in the

* Corresponding author. Tel.: +358 503175575.
*E-mail address:* jenni.viinamaki@helsinki.fi (J. Viinamäki).

http://dx.doi.org/10.1016/j.forsciint.2016.08.031
0379-0738/© 2016 Elsevier Ireland Ltd. All rights reserved.

vicinity of a chromophore. Chemiluminescent nitrogen detector (CLND) and evaporative light scattering detector (ELSD) have traditionally been used for quantification without reference standards as their response is relatively independent of the analyte [14,15]. CLND has been proven to be suitable for single-calibrant quantification of nitrogen containing drugs using caffeine for calibration [16]. However, CLND provides an equimolar response only with compounds containing nitrogen, and the response is proportional to the number of nitrogen atoms in a molecule [15]. With ELSD, the accuracy of single-calibrant quantification is improved when calibration is performed with chemically similar compounds [14,15].

Another universal detector, the corona charged aerosol detector (CAD) is a mass-dependent detector. The LC mobile phase is converted into droplets that are dried forming particles consisting of analyte molecules, and a stream of positively charged nitrogen collides with the particles. The charge is then transferred to the particles and measured. As the amount of analyte increases, the size of the particles increases as well. Consequently, the charge of the particle is increased along with particle size, generating a signal directly proportional to the quantity of the analyte present. Unlike UV detection, which is concentration-dependent, the signal of CAD does not depend on the concentration of the analyte in the eluent [17]. In the analysis of pharmaceutical compounds CAD was more sensitive than ELSD and approximately as sensitive as the UV detector [18]. The response to different analyte structures was more uniform with CAD than with ELSD [19]. As with MS and ELSD, the CAD response depends on the composition of the mobile phase but this phenomenon can be compensated by applying an inverse post-column gradient [20].

In our previous study, we developed and validated a quantitative method for straightforward monitoring of basic drugs in blood samples, based on ultra-high performance liquid chromatography with two consecutive detectors: a photodiode array detector (DAD) and a CAD [21]. This UHPLC-DAD-CAD method utilized the response ratio of DAD to CAD, which provided the additional identification efficiency required, while the high stability of identification and quantification allowed the use of facile historic calibration. In the present study, our objective was to assess the UHPLC-DAD-CAD method for concurrent quantification of drug metabolites using the parent drug for calibration. Consequently, we applied the validated parent drug calibration to retrospectively re-process the blood sample data gathered from 633 post-mortem cases to discover the distribution of metabolite to parent ratios and combined metabolite–parent concentrations for six toxicologically relevant drugs.

## 2. Materials and methods

### 2.1. Instrumentation and chemicals

The UHPLC-DAD-CAD method, including instrumentation, sample preparation and materials used, has been described earlier in detail [21]. Briefly, blood samples were extracted at a basic pH with a mixture of ethyl acetate and butyl acetate. The analytical column was a HSS C18 of 2.1 mm × 150 mm equipped with a HSS C18 precolumn of 2.1 mm × 5.0 mm, both with a particle size of 1.8 μm (Waters, Milford, MA). UHPLC separation was performed using a mobile phase gradient consisting of 0.1% aqueous solution of trifluoroacetic acid and methanol. Analyte detection was performed using the two consecutive detectors, DAD and CAD. UV spectra were collected over the range 210–400 nm at collection speed 20 point/s and the quantification wavelength was 230 nm. CAD was operated using a nitrogen pressure of 35 psi. Calibration samples were prepared in sheep whole blood, and historic one-point calibration on both detectors was used. Reference standards for drugs and their metabolites were obtained from pharmaceutical companies and they were of pharmaceutical purity.

### 2.2. Validation of the method

The UHPLC-DAD-CAD method has been previously validated using the primary calibration method with use of the reference standards [21]. In this study, a secondary calibration method using the parent drug as calibration standard was tested for 23 metabolites of 21 drugs for which the reference standards were available in the authors' laboratory (Table 1). Selection of the

**Table 1**
Comparison of metabolite quantification by CAD and DAD between ordinary calibration and calibration using parent drug.

| Parent drug | Metabolite | Linear range[a] [mg/L] | Calibration point [mg/L] | $R_t$ (metabolite) $-R_t$ (parent) [min] | Average quantification difference CAD [%][b] | Average quantification difference DAD [%][b] |
|---|---|---|---|---|---|---|
| Amitriptyline | Nortriptyline | 0.1–5 | 1 | 0.150 | 14.4 | 14.3 |
| Citalopram | Norcitalopram | 0.05–5 | 0.5 | 0.076 | 16.2 | 9.5 |
| Clobazam | Norclobazam | 0.1–5 | 1 | −0.515 | 14.3 | 21.8 |
| Clomipramine | Norclomipramine | 0.1–5 | 0.5 | 0.136 | 5.7 | 28.6 |
| Dextromethorphan | *O*-desmethyldextromethorphan | 0.05–5 | 0.5 | −2.103 | 18.4 | 35.9 |
| Dextropropoxyphene | Nordextropropoxyphene[c] | 0.05–5 | 1 | −0.579 | 7.7 | |
| Diltiazem | Nordiltiazem | 0.1–5 | 0.5 | 0.107 | 12.0 | 5.1 |
| Doxepin | Nordoxepin | 0.05–5 | 0.5 | 0.125 | 22.3 | 16.2 |
| Fluoxetine | Norfluoxetine | 0.1–5 | 1 | 0.012 | 13.0 | 20.2 |
| Hydroxyzine | Cetirizine | 0.05–5 | 0.1 | 0.487 | 24.9 | 6.1 |
| Levomepromazine | Norlevomepromazine | 0.1–3 | 0.5 | 0.143 | 1.1 | 7.7 |
| Mianserin | Normianserin | 0.1–3 | 0.5 | 0.205 | 7.3 | 25.2 |
| Mirtazapine | Normirtazapine | 0.05–5 | 0.5 | −0.176 | 14.5 | 17.8 |
| Olanzapine | Norolanzapine | 0.05–5 | 0.1 | 0.078 | 14.9 | 12.7 |
| Quetiapine | OH-quetiapine | 0.05–5 | 5 | −3.214 | 40.0 | 8.3 |
| Sertraline | Norsertraline | 0.05–5 | 0.5 | −0.176 | 2.6 | 12.4 |
| Sildenafil | Norsildenafil | 0.1–5 | 0.5 | 0.056 | 21.5 | 6.8 |
| Tramadol | Nortramadol | 0.05–5 | 1 | 0.691 | 3.9 | 17.1 |
| Tramadol | *O*-desmethyltramadol | 0.1–5 | 1 | −1.337 | 21.2 | 52.9 |
| Trimipramine | Nortrimipramine | 0.05–3 | 0.5 | 0.163 | 18.1 | 22.9 |
| Venlafaxine | Norvenlafaxine | 0.1–3 | 0.5 | 0.075 | 9.4 | 14.8 |
| Venlafaxine | *O*-desmethylvenlafaxine | 0.1–3 | 0.5 | −1.988 | 30.9 | 12.5 |
| Verapamil | Norverapamil | 0.05–1 | 0.5 | −0.021 | 19.4 | 12.3 |

[a] Based on secondary calibration with parent drug, LOQ being lowest point of linear range.
[b] Difference in metabolite quantification between ordinary one-point calibration using metabolite reference standard (primary calibration) and one-point calibration using parent drug reference standard (secondary calibration).
[c] Detectable only above 1.0 mg/L by DAD.

Download English Version:

https://daneshyari.com/en/article/6462637

Download Persian Version:

https://daneshyari.com/article/6462637

Daneshyari.com