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Quantitative determination of dobutamine in newborn pig plasma samples by HPLC–MS/MS

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ABSTRACT

A novel gradient reverse phase high performance liquid chromatography tandem mass spectrometry (HPLC/MS-MS) was performed as a method for the determination of dobutamine hydrochloride (DOB) in newborn pig plasma samples. It was developed and validated after optimization of sample treatment and various chromatographic and mass spectrometric conditions. Trimethoxydobutamine (TMD) was used as internal standard. Heptafluorobutyric acid (HFBA) and ethyl acetate were used for the treatment of plasma samples. The separation of dobutamine and internal standard was done using a Kinetex F5 (50 × 2.1 mm, 2.6 μm, 100 Å) analytical column. The mobile phase was a mixture of acetonitrile and HCOOH 0.01%. The column oven temperature was optimized at 40° C and the flow rate was 0.25 mL/min. DOB and TMD were detected by multiple reaction monitoring (MRM) mode in ESI+, using a cone voltage (CV) of 25 V and a collision energy (CE) of 25 eV. The weighted calibration curve ($1/x^2$) was found to be linear over the concentration range of 1–100 ng/mL ($r^2 > 0.999$). The limit of quantification (LLOQ) of the method was 1 ng/mL. The values of selectivity, carryover, LLOQ, linearity, accuracy, precision, matrix effect, stability and recovery obtained meet the acceptable range according to European Medicines Agency (EMA) and Food and Drug Administration (FDA) guidelines. The method was efficiently applied to quantify DOB in plasma samples from a pharmacokinetic/pharmacodynamic study in a disease model of newborn piglet.

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1. Introduction

The administration of drugs in vulnerable populations such as the neonatal should be supported by specific studies in this population aimed to ensure the efficacy and safety of the treatments. Notwithstanding the inclusion of children as subjects of study in clinical trials is hindered by ethical, economical and practical constrains [1–3]. As a consequence, the availability of drugs officially approved for their use in this population is limited, and the majority of the drugs are used in neonates in an off-label manner [4]. The use of suitable juvenile animal models to increase the knowledge on the drug and help in the selection of the optimal paediatric dosing regimens can be an alternative to avoid or reduce the number of studies and/or patients required for a clinical trial [5]. In this regard, the neonatal pig is one of the most popular species used as a juvenile model to perform pharmacokinetic/pharmacodynamic (PK/PD) studies in neonates because of its anatomical and physiological similarities with the human population [6,7].

Dobutamine (4-[2-[4-(4-hydroxyphenyl)butan-2-ylamino]ethyl]benzene-1,2-diol) (DOB), is an inotropic synthetic catecholamine with a strong β-adrenergic activity [8,9], currently used off-label in the treatment of cardiac insufficiency (hypotension) in preterm neonates [10]. There is a lack of information regarding the PK and PK/PD in neonates, and of the effective and safe doses for this group of population. The number of studies aimed to increase the knowledge on dobutamine PK/PD behavior in neonatal patients with cardiac insufficiency is limited, and few are the studies dealing with the quantitative determination of DOB in the neonatal population, either human or animal. The performance of studies in juvenile animals, as the one presented here, provides a valuable insight to gain knowledge on how the dose-concentration-response can be different in this population and to provide a better understanding of drug PK/PD behavior in neonates [11,12].

Several methods have been reported for the analysis of DOB in plasma using HPLC with electrochemical [13–15], fluorimetric [16–19] or photodiode array (PDA) detectors [20]. Published HPLC

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coupled to fluorimetric or PDA detection methods are characterized by quantification limits ranged from 5 ng/mL to 50 ng/mL, and the need of high sample volumes and long analysis run times. Only those methods which use electrochemical detectors are able to reach limits of quantification below 1 ng/mL and there is only one HPLC-electrochemical detection method with an acceptable sample volume for the neonatal population (0.1 mL) and short analysis time (4 min) [15]. Studies using mass spectrometry (MS) for the analysis of DOB in biofluids are scarce with the exception of multi-target tests aiming to detect numerous drugs, including DOB, in a single run [21,22]. These methods are not validated completely as they are used for the qualitative determination of dobutamine in the simultaeous screening of different drugs. Only the work reported by Keski-Hynnila et al. [23] was found for the specific analysis of DOB in biological samples using MS, but without a quantitative purpose.

The most common sample preparations for plasma samples containing dobutamine are protein precipitation (PPT), where acetonitrile (ACN) and methanol (MeOH) are the mainly used organic precipitant agents (PA), and liquid–liquid extraction (LLE) with methyl *tert*-butyl ether (MTBE) and solid phase extraction (SPE) (Table 1).

The present paper reports the development and validation of a fast and sensitive method by using HPLC with tandem MS detection for the quantitative determination of DOB in newborn pig plasma samples. The analytical method developed, which only requires 100 μL of plasma, has been successfully applied to the analysis of plasma samples obtained in a study that aimed to investigate the drug PK/PD behavior when intravenously (i.v.) administered in monotherapy at different doses to a newborn piglet model for hemodynamic insufficiency.

2. Material and methods

2.1. Instruments

Chromatographic method was carried out on an Alliance HPLC 2695 separation module (Waters, Milford, MA, USA) coupled to a tandem mass spectrometer Quattro Micro (Waters, Milford, MA, USA) equipped with an electrospray ionization source operating in positive mode (ESI+). Data acquisition was performed using MassLynx 4.0 software (Waters, Milford, MA, USA). A Kinetex F5 (50 × 2.1 mm, 2.6 μm, 100 Å) chromatographic column (Phenomenex, Torrance, CA, USA) was used as stationary phase. Sample centrifugation was performed using an Eppendorf 5424 centrifuge (Eppendorf, Hamburg, Germany), the extracts were evaporated using a TurvoVap evaporator (Caliper Life Sciences, Barcelona, Spain) and the sonication was made with an Elmasonic S 60 (H) (Elma Schidbaner GmbH, Singen, Germany).

2.2. Reagents and solutions

Dobutamine hydrochloride and isoproterenol (ISO) were purchased from Sigma-Aldrich (Saint Louis, USA), and trimethoxy-dobutamine hydrochloride (TMD), used as internal standard, from Toronto Research Chemicals (North York, Canada). Formic acid (HCOOH) and acetronitrile (ACN) from Merck (Darmstadt, Germany) and Romil (Cambridge, UK), respectively, were used for mobile phase preparation. Trifluoroacetic acid (TFA) from Panreac (Barcelona, Spain), methyl *tert*-butyl ether (MTBE) and methanol (MeOH) both from Romil (Cambridge, UK), ethyl acetate (EtOAc) from Merk (Darmstadt, Germany) and heptafluorobutyric acid (HFBA) from Sigma Aldrich were used for sample treatment optimization. Purified water from a Milli-Q Element A10 System

Table 1
Methodologies found in the bibliography for dobutamine analysis.

Reference	Matrix	Technique	Matrix volume (mL)	Sample treatment	Chromatogram run time (min)	Linear concentration range (ng/mL)	LLOQ (ng/mL)	Reproducibility (%RSD) and accuracy (%RE)	Recovery (%)
GE. Hardee et al. [11]	Equine plasma	HPLC/Fluorescence and Electrochemical detection	1	SPE	15	0.1–100	0.1	–	82–93
PH. Schwartz et al. [12]	Children plasma (from 0.13 to 16.6 years)	HPLC/Electrochemical detection	0.5	SPE	–	0.25–1000	–	<15 (intra-day)	–
H. Husseini et al. [13]	Human plasma	HPLC/Electrochemical detection	0.1	PPT LLE	4	0.1–600	1 (PPT) 100 (LLE)	<15 (inter-days)	–
H. Lingeman et al. [14]	Rat Plasma	HPLC/Fluorescence	0.2	LLE	10	10–600	14.5	<15 (intra-day)	81.1 ± 4.9
C. Leftourt et al. [15]	Human plasma	HPLC/Fluorescence	1	SPE	12	5–1000	5	Reproducibility 3.1–9.3 Accuracy 0.8–9.40	87.0 ± 2.3
J. Ahonen et al. [16]	Human plasma	HPLC/Fluorescence	–	LLE	–	0.2–200	20	<15 (intra-day)	–
DW. McKennon et al. [17]	Human plasma	HPLC/Fluorescence	–	–	–	25–300	10	–	–
R.Thippani et al. [18]	Rat Plasma	HPLC/PDA	0.9	PPT	10	50 – 2000	50	<15	98 ± 1.7
WH. Kwok et al. [19]	Horse Blood and Urine	UHPLC–MS	0.2 (plasma) and 0.02 (urine)	PPT (plasma) and dilution (urine)	12	–	–	14.3 (plasma) and 14.1 (urine)	40.5
T. Li et al. [20]	Animal Serum, Urine, Feed, Muscle and Liver Tissue	UPLC-Q-Orbitrap	2 (serum and urine) and 2 g (tissues)	PPT and SPE	15.5	0.02 – 500	0.383 (serum), 0.427 (urine), 0.86 (feed), 0.380 (muscle) and 0.420 (liver)	<15	72.1 ± 4.3 –108.9 ± 5.2
H. Keski-Hynnilä et al. [21]	Rat Urine and in vitro incubation mixtures	HPLC-QqQ	1 and 105 cells/cm^2	SPE and in vitro incubation	84	–	–	–	–

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