Development and Validation of a Dried Blood Spot LC-MS/MS assay To Quantify Furosemide in Human Whole Blood: An Approach to Improve the Design of PK Studies in Pediatric Population.

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Abstract: Furosemide remains to be one of the most widely used therapies in pediatric and neonatal intensive care and is a drug of choice in the long-term management of cardiac, pulmonary, and kidney diseases in children. Quantification of furosemide in neonates and small children require the availability of highly sensitive assays in small volume of sample. Here, we developed a highly sensitive LC-MS/MS method utilizing dried blood spots for determination of furosemide in small volumes of human whole blood (30µl). A 3 mm disc was punched from each DBS and extracted with methanol basified with ammonia containing diclofenac internal standard (IS). Chromatographic separation was carried out on ZORBAX SB C18 (100*4.6*3.5) column using an isocratic mobile phase mixture containing acetonitrile/0.2% acetic acid (70:30, V/V) at a flow rate of 0.6 ml/min. Detection involved a TSQ vantage in the negative ion mode. The assay was validated over the concentration range of 2.5-1250 ng/mL. Intra and inter assay precision values (% CV) were less than 11.0% while the accuracy was within ±15%. Furosemide is stable in the DBS samples at room temperature and in freezer at -40 ± 10°C for at least 60 days.

Key words: Dried blood spot, Furosemide, Hematocrit, LC-MS/MS, Pediatric

I. Introduction

Prescribing off label drugs is most common in children [1] as most drugs are developed on the basis of trials with adults. This practice places children at risk of adverse events and therapeutic failures because of the lack of appropriate safety, pharmacokinetic (PK), pharmacodynamic and efficacy studies [2,3]. The lack of trial is due to specific challenges in conducting studies in this population of children. Furosemide is an anionic acid derivative \((5\)-(aminosulfonyl)-4-chloro-2-[(2-furanylmethyl)amino]benzoic acid) belongs to a class of loop diuretics, used in pediatric patients to reduce edema in both acute and chronic cardiovascular, pulmonary, and kidney diseases [4,5]. Long-term furosemide therapy is used in infants with chronic lung disease or children with nephrotic syndrome or cardiac failure [6]. Furosemide is one of the off label drug and was listed as a highly priority drug requiring extensive pediatric investigation by National Institute of Child Health and Human Development (NICHD), part of HHS’ National Institutes of Health (NIH), in consultation with FDA and experts in pediatric research [7].

Several analytical methods including HPLC was widely used either with UV \([8-11]\) or fluorescence \([12, 13]\) detection for the estimation of furosemide in various biomatrieessuch as serum, plasma and urine. These methods used a high flow rate ranging from 1.5 mL/min to 3.5 mL/min with limit of quantitation ranging from 10 ng/mL to 50ng/mL. The developed LC-MS/MS methods found in the literature have used a high volume of plasma with liquid-liquid extraction [14] and some of the methods were developed in urine [15-16]. Sora et al [17] have proposed a method for simultaneous determination of furosemide with other diuretics. Many of the proposed methods have several limitations such as lack of sensitivity or requiring large volume of sample and practically these methods are not suitable in very low volume of pediatric blood samples. According to European Medicines Agency, for minimal risk studies blood loss for research trials should not exceed 1% of total blood volume at any given time or 3% of total blood volume over four weeks, making studies in pediatrics especially neonates difficult [18].

Dried blood spot (DBS) screening has been used as an alternative sample collection for pediatric purposes in 1960 [19] and was played an important role in neonatal screening of inborn errors of metabolism. Recently a few bioanalytical methods have been reported involving DBS-LC-MS/MS for pediatric population, using micro volume samples of capillary blood to measure drug levels with high precision [20-22]. The objective of this method was to develop and validate a LC-MS/MS assay to quantify furosemide on dried blood spots using low volumes of sample (30µl). This assay was specifically developed to apply for any pediatric pharmacokinetic trials and to overcome aforementioned regulatory concerns in pediatric blood sampling. This allows a snap shot for a complete time drug concentration profiles in pediatric patients.

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II. **Experimental**

### 2.1 Chemicals and materials:

Furosemide reference sample (99.0 % pure) and diclofenac (>97% pure) were purchased from Clearsynth Labs (P) Ltd (Mumbai, India). Their chemical structures were shown in Fig. 1. HPLC grade methanol and acetonitrile were purchased from JT Baker (Phillipsburg, USA). Acetic acid as an additive for LC-MS was purchased from Sigma aldrich (Bangalore, India). Water used for the LC–MS/MS analysis was prepared by using Milli Q water purification system procured from Millipore (Bangalore, India). FTA DMKP-A cards and Harris Uni-Core Punch, 3 mm were purchased from GE healthcare. The control human whole blood was procured from Deccan’s Pathological Lab’s (Hyderabad, India).

### 2.2 LC–MS/MS instrument and conditions:

An HPLC system (Shimadzu, Kyoto, Japan) consisting of a binary LC–20AD prominence pump, an auto sampler (SIL-HTc) and a solvent degasser (DGU–20A3) was used for the study. Aliquot of 20 µL of the extracted samples were injected onto the column, Agilent ZORBAX SB C18 (100*4.6*3.5), which was kept at 40 °C. An isocratic mobile phase consisting of a mixture of acetonitrile and 0.2% acetic acid in water (70:30 v/v) at a flow rate of 0.6 mL/min was used to separate the analyte from the endogenous components. Detection was carried out on TSQ Vantage triple quadrupole mass spectrometer (Thermo Scientific, USA) with a heated electro spray ionization (HESI-II) probe operated in negative ion mode at a spray voltage of 4.0 kv, capillary temperature of 270 °C and vaporizer temperature of 350 °C. Sheath and auxiliary gas pressures were set at 60 and 15 units respectively. Compound optimisation was done manually using Thermo TSQ Tune Master 2.1.0.1028 (Thermo Scientific, USA) by infusion 1µg/mL solutions of furosemide and IS separately into the mass spectrometer and the scan parameters were shown in Table1. Selective Reaction Monitoring (SRM) mode was used for data acquisition. Peak integration and calibration were carried out using LC Quan 2.5.2 software.

### 2.3 Preparation of Stock solutions of the analyte and IS:

Two different sets of primary stock solutions for furosemide were prepared by separate weighing, to get at a concentration of 1mg/mL in methanol and were stored at 2-8 °C for their stability. Each set of primary stock solutions were suitably diluted with methanol:water(50:50,V/V) for the preparation of working standard solutions of calibration curve (CC) and quality control (QC) samples. A working IS dilution (1000ng/mL) was prepared in methanol containing(0.1% ammonia solution, (25%))

### 2.4 Preparation of calibration curve standards and quality control samples:

Calibration samples of furosemide were prepared at concentration levels of 2.5, 5.0, 12.5, 25.0, 62.5, 125.0, 250.0, 625.0, 1000.0 and 1250.0 ng/mL as a single batch at each concentration levels. Similarly, quality control (QC) samples were also prepared as a bulk based on an independent weighing of standard drugs, at concentrations of 2.7 (LLOQ), 6.0 (LQC), 100.0 (MQC) and 1000 ng/mL (HQC) as a single batch at each concentration. Both calibration standards and Quality control samples were prepared in human K2 EDTA blood.

The final hematocrit in the prepared calibration standards and QC samples were about 0.45. A 30µl aliquot of each calibration standard and QC sample was spotted onto the DBS cards. The cards were left on the bench at ambient room temperature for overnight for complete drying of blood spots and then packed in a sealed plastic bag with small amount of desiccant.

### 2.5 Sample Extraction:

A 3-mm disc was punched from each dried blood spot sample using Harris Uni-Core Punch and transferred to individual 1.5ml eppendorf tubes. 400µl of IS working solution(1000ng/mL) was added to all samples, except matrix blanks to which 400µl of methanol (0.1% ammonia) was added. The tubes were capped tightly and gently mixed for 20minutes on Vortexer (Eppendorf mixmate) and then centrifuged at 14000g for 5min. 300µl of each extract was transferred into 5ml glass tubes and evaporated under nitrogen. The dried residue was dissolved in 100µl of mobile phase. 20µl aliquot of it was injected on to the column.

### 2.6 Method Validation

Validation of the method was carried out as per FDA guidelines [23] (Bioanalytical Method Validation published in May 2001).

#### 2.6.1. Selectivity, sensitivity and linearity:

The selectivity of the method was assessed by analyzing six different lots of blank human blood for interference at the retention time of furosemide and it’s IS. The area responses of the interfering substances or noise at the retention times of the furosemide and IS were acceptable, if the % interference was less than 20% of the mean response of the lowest standard in calibration curve point or LLOQ (n = 6). The area...
responses of the interfering substances or noise at the retention time of internal standard were acceptable, if the
% interference was less than 5% mean response of the internal standard areas in the 6 LLOQ samples.

The lower limit of quantitation(LLOQ) was defined as the lowest concentration of the calibration curve
that always exhibits an accuracy of ±20% of the nominal concentration and precision of ≤20%.More than five
separate assays each consisting of blank sample, a zero sample (matrix sample processed without analyte but
with internal standard), eight non-zero calibration standards in the range of 2.5-1250ng/mL were analysed. The
analyte/IS peak area ratio against nominal concentration of furosemide was used for a linear calibration
regression with a weighting factor of 1/x2.

2.6.2. Accuracy and precision:
Intra-day and inter-day precision and accuracy were assessed by analyzing QC samples at four
concentrations(2.7, 6.0, 100, 1000 ng/mL).Six replicates of each QC sample were analysed in the same batch
%CV and percent error were calculated for each set of replicated per batch to determine the intraday precision
and accuracy. Inter day precision and accuracy were determined at four different QC levels over four
consecutive days The acceptance criteria for accuracy should within ±15% standard deviation to their nominal
concentrations except for LLOQ, it should be ±20 and precision should within ≤15% relative standard deviation
(RSD) except for LLOQ, it should be ≤20%.

2.6.3. Recovery, matrix effect and dilution integrity
The recovery was calculated by analyzing extracted QC samples at three levels (LQC, MQC and HQC)
with that of the aqueous equivalents, which were having the same concentration of the QC samples prepared in
mobile phase without any extraction procedure. Similarly, the recovery of the IS was determined by comparing
the mean peak areas of the extracted QC samples for their IS area with the aqueous equivalent’s IS area. The
acceptance for the recovery was the RSD of the areas obtained for extracted and aqueous equivalents should be
≤15% individually at each level of QC samples and the RSD of the % recoveries for all of QC levels should be
≤15%. The recovery should be <115% at any level of concentration.
Matrix effect, expressed as IS normalized matrix factor (MF) was assessed by comparing the mean area
response of post-extraction spiked samples with mean area of aqueous samples (neat samples) prepared in
mobile phase solutions at LQC and HQC levels. The overall precision of the matrix factor was expressed as
coefficient of variation (CV). Matrix Factor = Mean peak response area ratio in absence of matrix ions
Peak response area ratio in presence of matrix ions
To demonstrate that a method is suitable for a DBS sample to quantify analyte concentration higher than the
ULOQ, the dilution integrity should be assessed. However, different from whole blood, plasma or serum which
can be diluted using the same matrix blank prior to analysis a DBS itself cannot be diluted. A simple approach is
to dilute the extracted dilution QC sample using one or more of extracted DBS zero samples(containing IS
only).The diluted QC sample was then analyzed along with the calibration standards. The obtained bias from the
diluted QCs in six replicates should be within ±15% of the nominal value and %CV<15%.

2.6.4 Impact of blood volume spotted on the DBS card:
To evaluate the effect of spotting volume on accuracy of the end result different spotting volumes of 10
and 20 µl were compared at LQC and HQC levels. These QC samples were analysed against calibration
standards prepared using 30µl of blood volume. A difference within ±15% of the nominal values would suggest
no significant effect of blood volume dappled on the DBS card.

2.6.5. Effect of hematocrit:
Influence of hematocrit on assay quantitation was measured by preparing two different concentrations
of furosemide at LQC and HQC level in whole human blood with a range of hematocrit values ranging from
0.25 to 0.75 for both the QC levels respectively. A difference within ±15% of the nominal values for the
measured analyte concentrations from the hematocrit QC samples against calibration standards that were
prepared in fresh blood with a hematocrit value of 0.45% is acceptable showing a negligible hematocrit effect.

2.6.6. Stability
Stability experiments were conducted to evaluate the furosemide stability at different storage and
processing conditions.Stability of furosimide in human DBS samples stored at room temperature and -40°C was
assessed over a period of 60 days. The concentrations of the stored samples were determined using freshly made
STDs and QCs. Autosampler stability, and reinjection reproducibility was also evaluated. Samples were
considered stable, if assay values were within the acceptance limits of accuracy should be ±15% of their
nominal concentrations and precision should be ≤15 RSD.
III. Results and Discussion

3.1. Mass spectrometry:  
Mass parameters were tuned in both “+” Ve and “−”Ve ionization modes. The more reproducible area was achieved in negative polarity mode for both analyte and IS. The data from SRM mode was considered to obtain better selectivity. The deprotonated [M - H] form of analyte and IS was the parent ion in the Q1 spectrum. The same was used as the precursor ion to obtain Q3 product ion spectra. The most sensitive mass transitions were monitored from m/z 328.9 to 284.8 for furosemide and from 293.8 to 249.9 m/z for diclofenac.

3.2. Method development:  
Separation was attempted using various combinations of Methanol, acetonitrile and buffer with varying contents of each component on different columns; C8 and C18 of different makes. The use of 0.2% acetic acid was useful in achieving optimum reproducible response. Mobile phase consisted of acetonitrile and 0.2% acetic acid at (70:30%, V/V) was found to be suitable, at which furosemide and IS were eluted at 1.9 min and 2.2 min respectively on ZORBAX SB C18 (100*4.6*3.5)column. The mobile phase drawn at a flow rate of 0.6 mL/min gave shorter run time of the chromatography. Methanol containing 0.1% ammonia, as an extraction solvent proved to be rugged and provided the cleanest samples. An appropriate internal standard will control for extraction, HPLC injection and ionization variability. After checking with several available compounds, finally diclofenac was found to be the best to serve as an internal standard.

3.3 Selectivity:  
The selectivity of the method was examined by analyzing extracted dried blank human blood samples from six different sources. Typical chromatograms of extracted blank human blood spot (Fig. 2A) and an extract spiked only with the IS (Fig. 2B). As shown in Fig. 2A, no significant direct interference in the blank was observed from endogenous substances in drug–free human blood at the retention time of the analyte and the IS. Similarly, Fig. 2B shows the absence of direct interference from the IS to the SRM channel of the analyte. Fig. 2C depicts a representative ion–chromatogram for the LLOQ sample (2.5 ng/mL).

3.4. Recovery:  
Methanol containing 0.1% ammonia solution(25%) as an extraction solvent proved to be rugged and provided the cleanest samples. The % recovery was more than 92.5% and the CV (%) was <12.9%.

3.5. Matrix effect and Sensitivity:  
There was no significant matrix effect observed in all six lots of human blood for the analyte at low and high QC level concentrations. The precision and accuracy for furosemide at LQC concentration were found to be 10.9% and 104.0%, and at HQC level they were 13.2% and 95.64%, respectively, the %CV of the IS-normalized matrix factor was less than 13.9%. Results revealed that no significant matrix effect was observed in all the six batches of human blood.

The lowest limit of reliable quantification for the analytes was set at the concentration of the LLOQ. The precision and accuracy of analyte at 2.5 ng/mL concentration was found to be 7.25% and 99.9%, respectively.

3.6. Linearity, Precision and accuracy:  
The ten-point calibration curve was found to be linear over the concentration range of 2.5-1250 ng/mL for furosemide. After comparing the weighting factor models at none, 1/X and 1/X0.9, the linear regression equation with weighting factor 1/X0.9 of the analyte to the internal standard concentration found to produce the best fit for the concentration–detector response relationship. The mean correlation coefficient of the weighted calibration curves generated during the validation was ≥ 0.99.

The results for intra–day and inter–day precision and accuracy are summarized in Table 2. The intra–day and inter–day precision deviation values were all within 15% of the relative standard deviation (RSD) at LQC, MQC and HQC levels, whereas within 20% at LLOQ QCs level. The intra–day and inter–day accuracy deviation values were all within 100 ± 15% of the actual values at LQC, MQC and HQC levels, whereas within 100± 20% at LLOQ QCs level. The results revealed good precision and accuracy.

3.7 Dilution integrity:  
Dilution integrity assessed at 2000 ng/mL with 2x and 4x dilution with blank zero sample was found to be within acceptance limit with precision(%CV) <9.0 and within 96.8 to 98.7 % of nominal values.

3.8 Stability studies:  
Table 3 shows the Autosampler stability of the sample extracts stored at 4°C for 55 hours.reinjection stability for 45 h. Long term stability was established for 60 days both at room temperature and at -40 ± 10°C.
3.9 Effect of blood volume spotted on the DBS card:
Furosemide concentration from spot volumes of 10, and 20µl was evaluated for demonstrating whether an exact spotting volume is required for accurate results. Table 4 shows both the spot volumes were comparable to those 30µl with %CV of ≤12.5 and within 85.8 to 111.4% of nominal values.

3.10 Effect of hematocrit:
Hematocrit values differ in pediatric samples [24], so attempts were made to establish the effect of hematocrit on DBS analysis. Table 5 shows that results with different hematocrit values ranging from 0.25% to 0.75%. Samples with lower hematocrit(0.25) resulted in higher deviation at both the concentrations tested.

IV. Figures and Tables

Figure 1: chemical structures of (A) furosemide and (B) diclofenac sodium

Figure 2: it shows typical chromatogram of furosemide (left panel) and IS (right panel) in human blank blood
[A], blood spiked with internal standard [B] and lower limit of quantification sample along with IS [C].

**Table 1: Mass Spectrometry Parameters**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Parent ion (m/z)</th>
<th>Product ion (m/z)</th>
<th>Collision Energy (V)</th>
<th>S lens (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furosemide</td>
<td>328.9</td>
<td>284.8</td>
<td>27</td>
<td>115</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>293.8</td>
<td>249.9</td>
<td>31</td>
<td>105</td>
</tr>
</tbody>
</table>

**Table 2: Precession and accuracy of intra and inter day analysis for the determination of furosemide in human blood.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Conc. Added (ng/mL)</th>
<th>Intra-day Precision and Accuracy</th>
<th>Inter-day Precision and Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(n=12; 6 from each batch)</td>
<td>(n=30; 6 from each batch)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Conc. found Mean±SD (ng/mL)</td>
<td>Mean±SD (ng/mL)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Precision (CV%)</td>
<td>Precision (%CV)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Accuracy (%)</td>
<td>Accuracy (%)</td>
</tr>
<tr>
<td>LLOQ</td>
<td>2.7</td>
<td>2.675 ± 0.283</td>
<td>10.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>99.1</td>
<td>2.660 ± 0.246</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>98.5</td>
</tr>
<tr>
<td>LQC</td>
<td>6.0</td>
<td>6.605 ± 0.406</td>
<td>6.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>110.1</td>
<td>6.575 ± 0.359</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>109.3</td>
</tr>
<tr>
<td>MQC</td>
<td>100.0</td>
<td>99.387 ± 5.940</td>
<td>5.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>99.4</td>
<td>100.292 ± 6.372</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100.3</td>
</tr>
<tr>
<td>HQC</td>
<td>1000.0</td>
<td>1095.672 ± 57.500</td>
<td>5.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>109.6</td>
<td>1103.227 ± 60.790</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>110.3</td>
</tr>
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</table>

**Table 3: Stability data for furosemide in human blood**

<table>
<thead>
<tr>
<th>Stability test</th>
<th>QC Spiked concentration (ng/mL)</th>
<th>Mean ± SD (ng/mL)</th>
<th>Precision (%CV)</th>
<th>Accuracy/ Stability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auto Samplera</td>
<td>6.0</td>
<td>6.539 ± 0.607</td>
<td>9.29</td>
<td>109.0</td>
</tr>
<tr>
<td>Re injectionb</td>
<td>6.0</td>
<td>5.919 ± 0.717</td>
<td>12.11</td>
<td>95.7</td>
</tr>
<tr>
<td>Long term</td>
<td>6.0</td>
<td>5.589 ± 0.375</td>
<td>5.69</td>
<td>108.9</td>
</tr>
<tr>
<td>Long term</td>
<td>1000.0</td>
<td>975.488 ± 52.089</td>
<td>5.34</td>
<td>89.7</td>
</tr>
<tr>
<td>6.0</td>
<td>1025.432 ± 37.318</td>
<td>3.64</td>
<td>102.5</td>
<td></td>
</tr>
<tr>
<td>6.0</td>
<td>6.416 ± 0.339</td>
<td>5.28</td>
<td>106.9</td>
<td></td>
</tr>
<tr>
<td>1000.0</td>
<td>1092.220 ± 67.950</td>
<td>6.22</td>
<td>109.2</td>
<td></td>
</tr>
</tbody>
</table>

a after 55h at 2–8°C; b after 45 h at 2–8°C; c after 60days at room temperature and in freezer -40±10°C

**Table 4: Effect of spotting volume on accuracy and precision of furosemide from extracted human blood**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Spotting Volume (µl)</th>
<th>QC Spiked concentration (ng/mL)</th>
<th>Mean ± SD (ng/mL)</th>
<th>Precision (%CV)</th>
<th>Accuracy %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>6.0</td>
<td>5.150 ± 0.339</td>
<td>6.58</td>
<td>83.8</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>6.0</td>
<td>6.683 ± 0.821</td>
<td>7.26</td>
<td>111.4</td>
</tr>
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</table>

**Table 5: Effect of hematocrit on accuracy and precision of furosemide from extracted human blood**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Hematocrit</th>
<th>QC Spiked concentration (ng/mL)</th>
<th>Mean ± SD (ng/mL)</th>
<th>Precision (%CV)</th>
<th>Accuracy %Diff</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.25</td>
<td>6.0</td>
<td>4.950 ± 0.342</td>
<td>6.90</td>
<td>82.5</td>
</tr>
<tr>
<td>2</td>
<td>0.35</td>
<td>6.0</td>
<td>5.972 ± 0.38201</td>
<td>4.82</td>
<td>79.3</td>
</tr>
<tr>
<td>3</td>
<td>0.45</td>
<td>6.0</td>
<td>1029.388 ± 20.449</td>
<td>1.99</td>
<td>103.0</td>
</tr>
<tr>
<td>4</td>
<td>0.55</td>
<td>6.0</td>
<td>1016.647 ± 66.147</td>
<td>6.51</td>
<td>101.7</td>
</tr>
<tr>
<td>5</td>
<td>0.65</td>
<td>6.0</td>
<td>1024.693 ± 83.996</td>
<td>8.20</td>
<td>102.5</td>
</tr>
<tr>
<td>6</td>
<td>0.75</td>
<td>6.0</td>
<td>982.496 ± 43.171</td>
<td>4.39</td>
<td>98.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1000.0</td>
<td>959.580 ± 102.221</td>
<td>10.65</td>
<td>96.0</td>
</tr>
</tbody>
</table>

V. Conclusion:

In summary, we have developed and validated a rapid, accurate, sensitive and rugged dried blood spot assay coupled with an LC-MS/MS for determination of furosemide in whole human blood. The analyte quantification was performed from small volumes of human blood requiring only 30µl, allowing an efficient use of limited blood samples in pediatric patients. Use of Hematocrit values between 0.25 to 0.75 gave acceptable precision accuracy values except for hematocrit value 0.25, unacceptable results were observed. It is believed...
that this DBS-LC-MS/MS based assay could be a highly useful assay in supporting clinical trials in paediatric patients.

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References