**Evaluation of pulsed electromembrane extraction for analysis of diclofenac and mefenamic acid in biological fluids**

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<td>Fotouhi, Lida; Alzahra University, Seidi, Shahram; K.N. Toosi University of Technology, Yamini, Yadollah; Tarbiat Modares University, Hosseini, Elham; Alzahra University,</td>
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</table>
Evaluation of pulsed electromembrane extraction for analysis of diclofenac and mefenamic acid in biological fluids

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Abstract

In the present study, electromembrane extraction (EME) and pulsed electromembrane extraction (PEME) coupled with high-performance liquid chromatography (HPLC) were compared for the extraction of two acidic drugs including diclofenac (DIC) and mefenamic acid (MEF). The effect of fundamental parameters on extraction efficiency of both EME and PEME were investigated. Under the optimized conditions, preconcentration factors in the range of 166 to 178 and 227 to 243 were obtained using EME and PEME, respectively. The limits of detection for DIC and MEF were obtained as 5.0 ng mL\(^{-1}\) using EME and 2.5 ng mL\(^{-1}\) by PEME. The dynamic linear ranges (DLRs) of both acidic drugs by EME were in the range of 20-250 ng mL\(^{-1}\) \((R^2 > 0.9970)\), whereas these ranges were 10-350 ng mL\(^{-1}\) \((R^2 > 0.9989)\) using PEME. The intra- and inter-assay precisions of the analysis were less than 5.36 and 6.64% by EME and 3.75 and 4.41 using PEME, respectively. The results showed that in comparison with EME, PEME is a more effective microextraction method, providing high extraction efficiencies in a short period of time. Finally, PEME was successfully used for extraction of DIC and MEF from urine and plasma samples. The calibration curves showed good linearity for urine and plasma samples with the coefficients of determination higher than 0.9965. The limits of detection were obtained 10 ng mL\(^{-1}\) for DIC in both urine and plasma and 10 ng mL\(^{-1}\) in urine and 15 ng mL\(^{-1}\) in plasma for MEF by PEME.

**Keywords:** Pulsed electromembrane/ Diclofenac/ Mefenamic acid/ Microextraction/ Plasma/ Urine.
1. Introduction

In an analytical process, clean-up is one of the main objectives of sample preparation in biological and environmental samples. Due to matrix complexity and low concentration of analytes, an effective extraction and purification approach is of vital importance prior to final analysis by chromatographic methods. Several different sample preparation procedures such as liquid-liquid extraction (LLE),¹ and solid-phase extraction (SPE),² have been proposed for extraction and purification approach.

LLE is a classical extraction method, which has been widely used for sample preparation before analysis. However, LLE is laborious, time consuming and requires large quantities of expensive and toxic organic solvents. SPE is another popular extraction and purification approach but it still involves large consumption of solvent and additionally suffers from clogging when handling complex real samples.³

During the last two decades many efforts have been focused on developing miniaturized sample preparation techniques for reduction of solvent usage and multistep extraction. These techniques can generally be classified as solid based, liquid based and membrane based microextraction techniques that are carried out as two or three phase extraction modes.

The latter case has become a dignified miniaturized extraction technique over the past decade. High clean-up and preconcentration of analytes as well as suitable selectivity are the major advantages that are provided by the membrane based microextraction techniques.

Hollow fiber liquid phase microextraction (HF-LPME) and EME are two important types of miniaturized membrane based extraction techniques introducing by Pedersen-Bjergaard et al.⁴⁻⁵ In both techniques, a piece of porous hollow fiber is used as support that is impregnated by a proper water-immiscible organic solvent. The extraction mechanism in HF-LPME is based on
passive diffusion of analytes\textsuperscript{6,7} whereas electrokinetic migration of ionized species, providing by applying an electrical field between two platinum electrodes locating into the sample solution and the lumen of the fiber, is the main extraction driving force in EME. Compared to passive diffusion, electrokinetic migration appears as a much more efficient transport mechanism due to providing high recovery in a short extraction time. Nowadays, EME has been developed for extraction of different basic and acidic analytes\textsuperscript{8-10}.

However, EME faces some problems such as serious instabilities in the analysis of real samples with high concentration levels of ionic species\textsuperscript{11}. In these samples, increasing of ion transport across the liquid membrane leads to Joule heating, increasing the current level through the liquid membrane and subsequently bubble formation due to electrolysis reaction\textsuperscript{11}. To overcome these drawbacks of the conventional EME, pulsed electromembrane extraction (PEME) was introduced by Rezazadeh \textit{et al.}, using a simple and inexpensive electronic device, which creates pulsed voltage in combination with a common DC power supply\textsuperscript{12}. In this technique, duration of the pulse is long enough for the migration of analytes from sample solution into the acceptor phase; but it is so short that the thickness of the boundary layer is minimized\textsuperscript{13}.

The purpose of this work is comparing of PEME with conventional EME to demonstrate the benefits of this new concept of electrically enhanced technique for extraction of two acidic drugs including DIC and MEF. These analytes are non-steroidal anti-inflammatory drugs which are used in the treatment of rheumatoid arthritis, post-trauma inflammation and other painful musculoskeletal disorders\textsuperscript{14}. Based on our knowledge, there is no report about extraction of acidic drugs from urine and plasma samples using PEME.
2. Experimental

2.1. Chemicals and materials

MEF and DIC were kindly donated by the Department of Pharmacy, Tehran University (Tehran, Iran). Methanol and acetonitrile were purchased from Caledon (Georgetown, Ont., Canada). 1-Octanol was purchased from Merck (Darmstadt, Germany). All used reagents were of analytical grade. The water used in the experiment was purified on a Milli-Q ultra-pure water purification system (Bedford, MA, U.S.A). The porous hollow fiber used for the SLM and for housing the acceptor solution was a PPQ 3/2 polypropylene hollow fiber from Membrana (Wuppertal, Germany) with an inner diameter of 600 µm, wall thickness of 200 µm, and pore size of 0.2 µm.

2.2. Standard solutions and biological samples

A stock solution containing 1.0 mg mL\(^{-1}\) of each analyte was prepared in methanol, stored at 4 \(^\circ\)C and protected from light. Working standard solutions were prepared by dilution of the stock solution in ultrapure water.

Urine samples including a healthy sample to construct calibration curves and calculations figures of merit were collected from volunteers with respect to human ethical guidelines. Also, the protocol was approved by an Internal Review Board. All urine samples were collected in clean and sterilized polyethylene bottles, sealed and stored at 4 \(^\circ\)C before extraction. For extraction, 1.0 mL of each urine sample was diluted to 5.0 mL with ultrapure water and its pH was adjusted by dropwise addition of NaOH solutions.

Plasma samples (Blood groups: O\(^+\)) were obtained from the Iranian Blood Transfusion Organization (Tehran, Iran) and stored at -20 \(^\circ\)C prior to use. Frozen plasma sample thawed and
was allowed to reach room temperature. One milliliter of the plasma sample was diluted to 5 mL with ultra-pure water and its pH was adjusted by dropwise addition of NaOH solutions.

2.3. HPLC analysis

Chromatographic separation of MEF and DIC was carried out on a Young Lin HPLC consisting of a YL9100 HPLC pump (Cambridge, England), a six-port two-position Rheodyne HPLC valve (Oak Harbor, Washington, U.S.A) with a 20 µL sample loop and equipped with a Y19120 HPLC UV-Vis detector. Chromatographic data were recorded and analyzed using Power Stream software (version 3.2). A C18 column (15 cm × 4.6 mm, with particle size of 5 µm) from Hichrom (Berkshire, England) was applied to separate the analytes under isocratic elution conditions. A mixture of 10 mmol L\(^{-1}\) acetate buffer (pH 5.2) and acetonitrile (50:50, v/v) with a flow rate of 1.0 mL min\(^{-1}\) was used as the mobile phase. The injection volume was 20 µL for all of the standards and the samples, and detection was performed at wavelength of 285 nm.

2.4. Equipment for EME and PEME

The same setups were used for both PEME and conventional EME except a home-made pulse-generator that was utilized during PEME. A 7.5 mL glass vial was used as sample compartment in both extraction methods. The electrodes used in this work were platinum wires with diameters of 0.5 and 0.2 mm for cathode and anode, respectively, which were obtained from Pars Platin (Tehran, Iran). The electrodes were coupled to a power supply model PTS 1002 with a programmable voltage in the range of 0–300 V and with a current output in the range of 0–2.5 A from Akhtarian (Tehran, Iran). A home-made pulse generator was used to set the pulse duration and outage period with a timer in the range of 1 s to 10 min. During the extraction, the EME unit was stirred at a stirring speed range of 0–1250 rpm by a heater-magnetic stirrer model 3001 from Heidolph (Kelheim, Germany) using a 5-mm × 3-mm magnetic bar. A 25 µL microsyringe
model 702 NR from Hamilton (Bonaduz, Switzerland) was employed during extraction procedure and also to inject the extracted analyte into the HPLC. All of the pH measurements were made using an 827 Metrohm pH meter (Herisau, Switzerland).

2.5. Extraction procedure for PEME and EME

The hollow fibers were cut into small segments with length of 7.5 cm and all the experiments were conducted at room temperature. DIC and MEF were spiked at the concentration of 100 ng mL\(^{-1}\) during optimization process. In both EME and PEME, 7 mL of alkaline sample solution (containing 1 mmol L\(^{-1}\) NaOH) as a donor phase (DP) was filled into the 7.5 mL glass vial. 1-octanol was used as organic membrane solvent and the lumen of the fiber was filled with about 20 \(\mu\)L basic solution containing 50 mmol L\(^{-1}\) of NaOH.

In EME, the platinum anode was introduced into the lumen of the fiber. The fiber containing the anode, together with the SLM and the acceptor solution were afterwards directed into the sample solution. The platinum cathode was led directly into the sample solution. The electrodes were subsequently coupled to the power supply. In the case of PEME, a home-made electrical device was located between platinum electrodes and power supply to generate pulse voltages. Electrical potentials of 40 and 60 V were applied during extraction by EME and PEME, respectively for a predetermined period of time. After the extraction was completed, the acceptor solution was collected by a microsyringe and injected into the HPLC instrument for further analysis. The preconcentration factors (PF) and relative recoveries (RR\%) were calculated based on the previous papers.\(^{15}\)

3. Results and discussions
Conventional EME was performed by applying continues voltage over a determined extraction time while the total extraction time of PEME consists of a “pulse duration or ON” during which the voltage is applied and an “outage period or OFF” defined as the time when the voltage is not applied. Both EME and PEME methods were carried out for extraction of two acidic drugs (MEF and DIC) to compare the advantages of EME and PEME.

To obtain the maximum extraction recoveries for determination of MEF and DIC, the effective parameters including, composition of the organic solvent (SLM), composition of donor and acceptor phases’, extraction time, applied voltage, stirring rate, and duration of the pulse and outage periods were optimized. All optimizations were done in ultrapure water.

3.1. The organic liquid membrane

According to the earlier findings, the chemical nature of the supported liquid membrane is highly critical for the success of EME. There are specific requirements for a solvent to be used as a SLM in EME. 1-Octanol has been the best candidates for acidic drugs in EME up to now. Therefore, 1-octanol was chosen as the organic solvent for SLM.

3.2. Applied voltage and extraction time

The main driving force for migration of the analytes across liquid membrane is provided by the electrical field. Strength of the electrical field is dependent on the applied voltage, and the voltage in turn affects the flux of analytes. Therefore, applied voltage is one of the most important parameters that should be regarded. Voltage and time are two parameters that act in parallel ways. Both time and voltage directly increase the flux of ions and thus increase extraction recovery; but there is an antagonistic effect when they are simultaneously considered, thus an increase in extraction time limits the voltage and vice versa. To obtain the optimum extraction voltage and time, these parameters were considered at the same time. For this purpose,
the extraction of both MEF and DIC was studied in different EME durations and electrical potential differences ranging from 5 to 20 min and 20 to 80 V, respectively. The results are demonstrated in Fig. 1A and B, respectively.

Similar experiments were designed to scrutinize the effect of voltage on the extraction of acidic analytes in PEME while the pulse durations (ON) and the outage periods (OFF) were considered constant as 10 s and 5 s, respectively (Fig. 1C and D). As shown in Fig. 1, the best results were obtained using application of electrical potentials of 40 and 60 V for the drugs by means of EME and PEME approaches, respectively.

On the other hand, both methods require sufficient time to reach the equilibrium; therefore, it is expected that the amounts of extracted analytes in the acceptor phase are increased by increasing the extraction time. As shown in Fig. 1, the peak area was increased by rising the extraction time up to 15 min, reached to a maximum at this time and showed a decline afterwards. It may due to gradual loss of the organic solvent that occurs at long extraction times. Thus time duration of 15 min was chosen as optimum extraction time to obtain the best extraction recoveries for drugs of interest by EME and PEME.

3.3. Investigation of duration of the pulse (ON) and outage (OFF) periods in PEME

It has been shown that pulsed electromembrane extraction increases the system stability by decreasing the thickness of double layer at the interfaces and improves extractability by eliminating this mass transfer barrier. In each pulse of PEME, voltage is applied for a relatively short time which is long enough for the transportation of analytes into the acceptor phase. During the outage period, the accumulated ions at the interfaces of SLM with both donor and acceptor phases are dispersed again throughout the stirring sample solution.
The effects of ON and OFF durations on the extraction deficiencies of DIC and MEF are shown in Fig. 2. For PEME, pulse durations were designed based on applying 60 V DC voltage in 15 min as the optimum voltage and extraction time. The ON and OFF durations were 5, 15, 25 s and 2, 6, 10 s, respectively. Results in Fig. 2 illustrate that the maximum extraction efficiencies of DIC and MEF are obtained by selection of 15 s and 6 s as the optimum ON and OFF periods, respectively.

3.4. Effect of salt in EME and PEME

According to the previous studies on EME, the presence of high contents of ionic species leads to an increase in the value of ion balance (χ) which defined as the ratio of the total ionic concentration in the sample solution to that in the acceptor phase. Indeed, increasing the concentration of the other ions into sample solution increases the competition among target analytes and interfering ions which in turn decreases the flux of target analytes across the SLM. Also, by increasing the concentrations of ions into donor phase, the numbers of ions across through the SLM are increased which consequently leads to increasing of the friction among ions and the organic solvent, excessive heat formation (Joule heating), and instability of SLM. This phenomenon can cause to puncture of SLM and spark generation between platinum electrodes in some cases. The effect of salt content in both EME and PEME was investigated by addition of sodium chloride into sample solution in the range of 1 to 5 mol L\(^{-1}\). High concentration levels of NaCl were selected to show sever electrolysis reactions and consequently fluctuation problems into acceptor phase which are resulted at high ionic strength. According to our observation, the stability of SLM in both EME and PEME system was severely affected by the salt content, so that high current levels (at the range of mA) were passed through the system at salt
concentrations upper than 2 mol L$^{-1}$. This caused to considerable fluctuations in the volume of acceptor phase due to electrolysis reactions, so that the final volume of the acceptor phase was reached less than 10 µL in some experiments. At low salt concentrations, PEME showed better stability of SLM and consequently much less electrolysis and fluctuations into acceptor phase. Finally, the negative effect of salt in both EME and PEME was revealed based on the increasing of SLM instability, amount of electrolysis reactions and fluctuations of acceptor phase during the extraction procedure. Therefore, electrical migration of the analytes would be more efficient at the absence of salt and all of the subsequent experiments were performed at such condition.

3.5. The effect of stirring rate in EME and PEME

Stirring of sample solution enhances diffusion of analytes by accelerating the mass transfer in donor phase and reducing the thickness of the Nernst’s diffusion film around the interface between the sample solution and SLM. The results showed that the peak areas increased by increasing of the stirring rate up to 1250 rpm for both PEME and EME methods.

3.6. Effect of pH of donor and acceptor phases in EME and PEME

In the following optimization process, the pH amounts of sample solution and acceptor phase were considered for extraction of DIC and MEF by both methods. It was found that the flux of the analytes is increased by decreasing the ion balance.\textsuperscript{17} Ion balance is mainly determined by the pH values of sample solution and acceptor phase. Sample solution should be basic enough, so that the acidic analytes carry a net negative charge to be enabled to migrate toward the anode in an electrical field. In the case of acceptor phase, decreasing of pH increases protonation probability of the analytes and accelerates their back-diffusion possibility to the SLM. On the other hand, increasing the pH of acceptor phase increases the release rate of acidic analytes into acceptor solution at the organic phase/acceptor phase interface and consequently increases the
extraction efficiency. However, there are some limitations for application of high concentration
of NaOH as the acceptor phase such as increasing the risk of bubble formation and fluctuation in
the acceptor phase volume.

To obtain the optimum pH values for donor and acceptor phases, the effects of these parameters
were investigated for both EME and PEME. For this purpose, the concentrations of NaOH in
both phases were changed in the range of 1 to 50 mmol L$^{-1}$, simultaneously. The results for EME
and PEME are shown in Fig. 3. As can be seen, similar behaviors were observed for both DIC
and MEF by EME and PEME. Finally, 1.0 and 50 mmol L$^{-1}$ of NaOH were chosen as the
optimum concentrations in donor and acceptor phases for both EME and PEME, respectively.

**Fig. 3**

3.7. Method performance

Figures of merit of the proposed method including limit of detection (LOD), linearity, PF and
intra- and inter-assay precision (RSD%) were evaluated for extraction of DIC and MEF. The
results of PEME in ultrapure water were compared with those acquired by EME (Table 1). As
can be seen in Table 1, PEME shows lower LODs, higher PF values and better repeatability and
reproducibility indicating its more extraction efficiency. This fact can be attributed to the
increasing of SLM stability by decreasing the thickness of double layer which is provided by
applying a pulse voltage during extraction procedure. Finally, the performance of PEME for
extraction of DIC and MEF was studied in drug-free urine and plasma samples. The results are
shown in Table 2 indicating suitable ability of PEME in biological fluids.

**Tables 1 and 2**

3.8. Analysis of DIC and MEF in urine and plasma samples by PEME
In order to study the influence of the biological fluids, PEME was applied for extraction and analysis of DIC and MEF from human plasma and urine samples. It was reported that the electrical potential in EME system can act as a powerful force for breaking and decreasing of analyte-protein binding.\textsuperscript{18} Therefore, no pretreatment was used for extraction of MEF and DIC in human plasma. The preparation steps of real samples were performed according to section 2.5. At first, non-spiked plasma and urine samples were analyzed by PEME under optimal conditions. Afterwards, different amounts of the drugs were added to real samples and extraction procedure was performed to calculate the relative recoveries. Table 3 shows that the results of each real sample obtained by the proposed method are in satisfactory agreement with the spiking amount. According to literature, after oral administration of 250 mg, three times a day for four days to 10 subjects, peak plasma concentrations of 0.3 to 2.4 g L\textsuperscript{-1} (mean 0.9) were reported 2 h after the morning dose.\textsuperscript{19} Therefore, the proposed method can easily detect the trace amount of DIC and MEF in real samples. Typical chromatograms obtained using PEME from drug-free urine (A) and plasma (B) samples after (a) and before (b) spike with DIC and MEF at concentration of 100 ng mL\textsuperscript{-1} were shown in Fig. 4.

The present method was compared with the other methods in terms of validation and precision (Table 4). As can be deducted, the method is quite comparable to those mentioned in Table 4.

4. Conclusions

This work was the first comparison of EME with PEME for analysis of acidic drugs. The major reason for instability problems in EME is current increasing. The electrical current of the system increases by increasing the applied voltage. On the other hand, accumulation of ions is occurred

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at the SLM interfaces by means of voltage application which causes to an increase in Joule heating and a decrease in the electrical resistance of the SLM. Therefore, even by applying low voltages for a long time, the resistance of the system is gradually reduced. Instability problems are more critical in systems with lower electrical resistance, since they hardly could endure high voltages and sparking may observe.\textsuperscript{20} This issue is more critical for extraction of acidic analytes because the best organic solvents for extraction of acidic analytes are linear alcohols such as 1-octanol with relatively considerable electrical conductivity.\textsuperscript{9}

Keeping these points in mind, a series of experiments were conducted for the first time to provide comparative results for extraction of two acidic analytes using EME and PEME. The different factors influencing the extraction efficiency of MEF and DIC by both EME and PEME were studied and optimized. The obtained results showed that PEME can reduce the instability problems of EME. This issue led to better repeatability and reproducibility values for extraction of MEF and DIC by PEME in comparison with EME. Moreover, better extraction efficiencies were observed for target acidic drugs by PEME attributing to the elimination of ion accumulation around the SLM interfaces in each outage period.

**Acknowledgments**

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**References**

5227-5233.


Figures caption

Fig. 1. Simultaneous investigation of time–voltage on extraction efficiency of (A and C) 100 ng mL$^{-1}$ DIC, (B and D) 100 ng mL$^{-1}$ MEF by EME (A and B) and PEME (C and D). One and 50 mmol/L of NaOH as DP and AP, respectively; pulse duration of 10 s and outage period of 5 s for PEME; stirring rate of 1250 rpm; 1-octanol as SLM.

Fig. 2. Simultaneous optimization of pulse duration (ON) and outage period (OFF) of (A) 100 ng mL$^{-1}$ DIC and (B) 100 ng mL$^{-1}$ MEF. Conditions as Fig. 1 except DC voltage of 60 V and extraction time of 15 min.

Fig. 3. Simultaneous optimization of concentration of NaOH in donor and acceptor phases of (A and C) 100 ng mL$^{-1}$ DIC, (B and D) 100 ng mL$^{-1}$ MEF by EME (A and B) and PEME (C and D). Conditions for EME as Fig. 1 except DC voltage of 40 V and extraction time of 15 min and for PEME as Fig. 2 except pulse duration of 15 s and outage period of 6 s.

Fig. 4. Chromatograms obtained using PEME from drug-free (A) urine and (B) plasma samples after (a) and before (b) spike with DIC and MEF at concentration of 100 ng mL$^{-1}$.
Table 1
Comparison of the performance of PEME with conventional EME applied for the extraction and determination of DIC and MEF in ultrapure water.

<table>
<thead>
<tr>
<th>Method</th>
<th>Analyte</th>
<th>LOD (ng mL⁻¹)</th>
<th>Linearity (ng mL⁻¹)</th>
<th>R²</th>
<th>PF</th>
<th>RSD%</th>
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</thead>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Inter</td>
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<td>EME</td>
<td>DIC</td>
<td>5.0</td>
<td>20-250</td>
<td>0.9973</td>
<td>178</td>
<td>5.14</td>
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<tr>
<td></td>
<td>MEF</td>
<td>5.0</td>
<td>20-250</td>
<td>0.9970</td>
<td>166</td>
<td>5.36</td>
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<td>PEME</td>
<td>DIC</td>
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<td>10-350</td>
<td>0.9990</td>
<td>243</td>
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<tr>
<td></td>
<td>MEF</td>
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<td>10-350</td>
<td>0.9989</td>
<td>227</td>
<td>3.75</td>
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Table 2
Figures of merit of PEME for DIC and MEF in urine and plasma samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Analyte</th>
<th>LOD (ng mL⁻¹)</th>
<th>Linearity (ng mL⁻¹)</th>
<th>R²</th>
<th>PF</th>
<th>RSD% Inter</th>
<th>RSD% Intra</th>
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<tr>
<td>Urine</td>
<td>DIC</td>
<td>10</td>
<td>30-350</td>
<td>0.9978</td>
<td>89</td>
<td>6.50</td>
<td>7.60</td>
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<tr>
<td></td>
<td>MEF</td>
<td>10</td>
<td>30-350</td>
<td>0.9968</td>
<td>114</td>
<td>6.14</td>
<td>7.15</td>
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<tr>
<td>Plasma</td>
<td>DIC</td>
<td>10</td>
<td>30-250</td>
<td>0.9979</td>
<td>81</td>
<td>7.05</td>
<td>8.37</td>
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<tr>
<td></td>
<td>MEF</td>
<td>15</td>
<td>30-250</td>
<td>0.9974</td>
<td>104</td>
<td>6.80</td>
<td>7.86</td>
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### Table 3
Determination of DIC and MEF in urine and plasma samples using PEME-HPLC-UV.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$C_{\text{Initial}}$ (ng mL$^{-1}$)</th>
<th>RR%</th>
<th>RSD% (n=3)</th>
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<tr>
<td></td>
<td>DIC</td>
<td>MEF</td>
<td>DIC</td>
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<tr>
<td>Urine 1</td>
<td>40</td>
<td>n.d</td>
<td>90.4$^b$</td>
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<tr>
<td>Urine 2</td>
<td>n.d</td>
<td>144.8</td>
<td>93.7$^b$</td>
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<td>Urine 3</td>
<td>n.d</td>
<td>n.d</td>
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<td>Plasma</td>
<td>n.d</td>
<td>n.d</td>
<td>92.1$^c$</td>
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$^a$ n.d: not detected.

$^b$ 40 ng mL$^{-1}$ of DIC and 140 ng mL$^{-1}$ of MEF were added in urine 1 and urine 2, respectively to calculate relative recovery percent (RR%).

$^c$ 100 ng mL$^{-1}$ of DIC and MEF were added in drug free urine and plasma samples to calculate RR%. 
Table 4
Comparison of the proposed method with other reported methods for determination of DIC and MEF.

<table>
<thead>
<tr>
<th>Method/instrumentation</th>
<th>LOD (ng mL⁻¹)</th>
<th>DLR (ng mL⁻¹)</th>
<th>R²</th>
<th>RSD%</th>
<th>Ref.</th>
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<tr>
<td>LLE/HPLC-UV</td>
<td>25.0</td>
<td>25-2000</td>
<td>0.998</td>
<td>-</td>
<td>21</td>
</tr>
<tr>
<td>HFME/EC-UV</td>
<td>-</td>
<td>100-2500</td>
<td>-</td>
<td>-</td>
<td>22</td>
</tr>
<tr>
<td>EME/GC-MS</td>
<td>0.26</td>
<td>1.1-200</td>
<td>0.982</td>
<td>13</td>
<td>23</td>
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<tr>
<td>SPE/HPLC-UV</td>
<td>-</td>
<td>1-200</td>
<td>0.999</td>
<td>-</td>
<td>24</td>
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<td>SPMTE/HPLC-UV</td>
<td>5.7</td>
<td>10-10000</td>
<td>-</td>
<td>-</td>
<td>25</td>
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<tr>
<td>EME/HPLC-UV</td>
<td>5.0</td>
<td>8-500</td>
<td>-</td>
<td>14.5</td>
<td>26</td>
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<tr>
<td>PEME/HPLC-UV</td>
<td>10b</td>
<td>30-350b</td>
<td>0.996b</td>
<td>&lt;7.6b</td>
<td>This work</td>
</tr>
<tr>
<td>PEME/HPLC-UV</td>
<td>10, 15c</td>
<td>30-250c</td>
<td>0.997c</td>
<td>&lt;8.37c</td>
<td>This work</td>
</tr>
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a Liquid-liquid extraction, Hollow-fiber microextraction, Electrophorese ultraviolet detection, Gas chromatography mass spectroscopy, Solid-phase extraction, Solid-phase membrane tip extraction
b Urine
c Plasma
Fig. 1

A

B

C

D

Analytical Methods
Fig. 2
Fig. 3
Fig. 4

A

DIC

MEF

B

DIC

MEF