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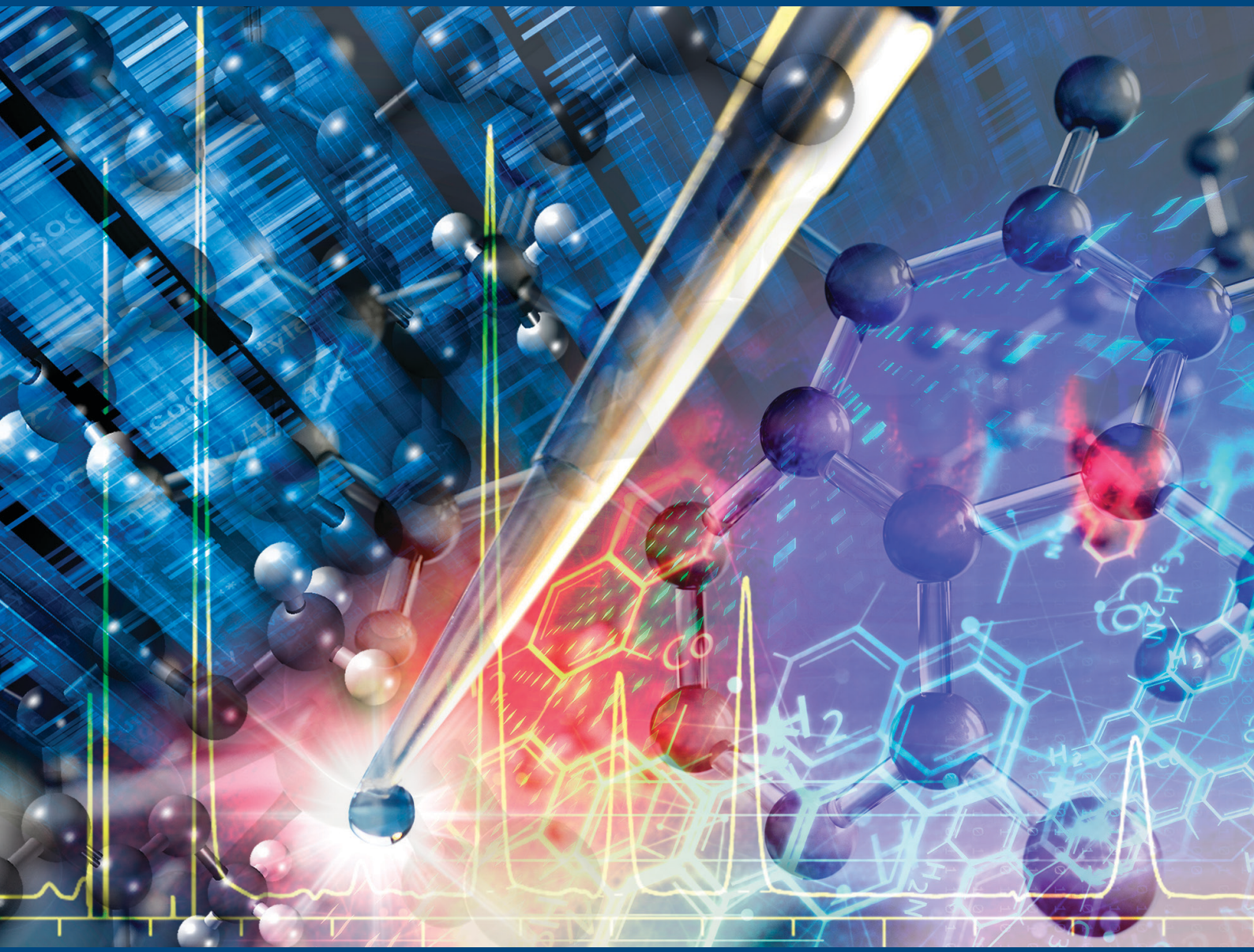

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RESEARCH ARTICLE

Optimization of generic conditions for electromembrane extraction of basic substances of moderate or low polarity

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Generic electromembrane extraction (EME) methods were developed and optimized for basic analytes of moderate or low polarity, employing prototype conductive vial EME equipment. Two generic methods, B1 and B2, were devised for mono- and dibasic compounds with distinct polarity windows: $2.0 < \log P < 6.0$ for B1 and $1.0 < \log P < 4.5$ for B2. In B1, 10 μL of 2-nitrophenyl octyl ether served as the liquid membrane, while B2 utilized 10 μL of 2-undecanone. Both methods involved the acidification of 125 μL of human plasma samples with 125 μL of sample diluent (0.5 M HCOOH for B1 and 1.0 M HCOOH for B2). The acceptor phase consisted of 250 μL of 100 mM HCOOH. Extraction was conducted for 30 min with agitation at 800 rpm, employing an extraction potential of 100 V for B1 and 50 V for B2. A set of 90 pharmaceutical compounds was employed as model analytes. Both B1 and B2 demonstrated high recoveries (40%–100%) for the majority of model analytes within their respective polarity windows. Intra-day precision was within 2.2% and 9.7% relative standard deviation. Both extraction systems exhibited stability in terms of current, matrix effect values were between 90% and 109%.

KEYWORDS
basic analytes, electromembrane extraction, generic methods, liquid-phase microextraction

1 | INTRODUCTION

Electromembrane extraction (EME) employs an electrical field to facilitate the extraction of charged analytes from a sample solution, across a liquid membrane, and into an aqueous acceptor solution. With only 1–10 μL of organic solvent as the liquid membrane, EME minimizes solvent consumption, aligning closely with sustainability and eco-friendliness. Furthermore, acceptor solutions are aqueous, enabling direct analysis by liquid chromatography-mass spectrometry (LC-MS) and related techniques, eliminating the need for laborious evaporation and reconstitution steps. EME offers unparalleled selectivity, influenced by factors such as the direction and magnitude of the electrical field, the composition of the liquid membrane, and pH adjustments.

While EME has gained substantial interest [1–3], it has been conducted on various laboratory-built systems under

Article Related Abbreviations: EME, electromembrane extraction; ETN, Extraction Technologies Norway; LC-MS, liquid chromatography-mass spectrometry; ME, matrix effect; NPOE, 2-nitrophenyl octyl ether; UHPLC-UV, ultra-high-performance liquid chromatography-ultraviolet.

a multitude of experimental conditions [4–8]. The transition of EME from research to a broadly applicable microextraction technique necessitates standardized and commercial equipment and the development of generic methods. Three-dimensionally-printed devices have been developed recently and address standardization [9]. Furthermore, commercial EME equipment has recently emerged [10], with vials designed for samples and acceptors composed of conductive polymer. With this technology, the electrical field is coupled through the vials. However, the development of generic methods remains a pivotal step.

Recently, a system of generic EME methods was presented [11]. This conceptual framework comprises a limited set of recommended extraction conditions, selected based on the charge (z) and polarity (log *P*) of the target analyte. The development of generic EME systems primarily concerns the selection of suitable liquid membranes. These membranes must satisfy several criteria, including efficient extraction within defined z and log *P* ranges (extraction windows), stability during extraction, and minimal current during operation. Achieving these objectives necessitates a delicate balance between the hydrophobicity of the liquid membrane and its molecular interactions with the analytes. Additionally, the very low water solubility of the liquid membrane is a crucial factor, as solvents with solubility exceeding 0.5 mg/mL can compromise system stability by leaking into the sample and acceptor phases. Electrolysis, an issue at higher currents, is also considered, particularly for complex matrix samples.

Obviously, a comprehensive description of the methodology employed in EME includes information about the liquid membrane (organic phase). However, the original sample is typically mixed with a sample diluent before extraction, which necessitates details about the type and volume of the sample, along with the composition and volume of the sample diluent. Furthermore, the composition and volume of the acceptor need to be specified, along with the key operational parameters including the extraction potential, agitation rate, and extraction time.

In previous research, liquid membranes specifically designed for the generic methods referred to as B1 and B2 were introduced for the extraction of mono- and dibasic analytes within the polarity range of $2.0 < \log P < 6.0$ and monobasic analytes within the polarity range of $1.0 < \log P < 4.5$, respectively [11]. For the generic method B1; 2-nitrophenyl octyl ether (NPOE) was selected as the ideal liquid membrane, while 2-undecanone was designated for use in B2.

The present study is dedicated to the comprehensive investigation and optimization of the sample, acceptor, and operational parameters in conjunction with NPOE and 2-undecanone. Our objective is to finalize the development of generic EME methods tailored for basic analytes possessing low or moderate polarity; the method with NPOE termed B1 and with 2-undecanone termed B2. Furthermore, this research aims to serve as a valuable guideline and reference for forthcoming method development.

2 | MATERIALS AND METHODS

2.1 | Chemicals

Methanol (LC-MS-grade), acetonitrile (LC-MS-grade), hydrochloric acid (37%), and phosphoric acid (85%) were purchased from Merck. NPOE, 2-undecanone, formic acid, acetic acid, citric acid, disodium hydrogen phosphate, trifluoroacetic acid, metoprolol, pethidine, oxprenolol, noscapine, droperidol, promazine, hydroxyzine, chlorpromazine, thioridazine, and meclizine were purchased from Sigma–Aldrich. Ultrapure water was provided by a Milipak (0.22 μm filter) Integral 3 purification system (Milli-Q).

2.2 | Plasma samples

Drug-free human plasma samples were obtained from Oslo University Hospital and stored at −28°C. The pharmaceutical substances metoprolol, pethidine, oxprenolol, noscapine, droperidol, promazine, hydroxyzine, chlorpromazine, thioridazine, and meclizine with log *P* ranging from 1.0 to 6.5 were used as model analytes (Figure S1). A stock-mixed standard solution containing 1 mg/mL of each model analyte was prepared by dissolving the substances in methanol. The stock mixed solution was split into aliquots and stored at −28°C. The stock mix solution was utilized to spike human plasma samples. Drug-free plasma was diluted 1:1 in buffer and spiked simultaneously. The acidification of the sample served to protonate basic analytes. Buffers in different concentrations of 10, 25, and 100 mM within the pH range of 1.0–3.4 were prepared. The pH in the donor and acceptor phases was measured with pH indicator strips (Merck KGaA).

2.3 | Equipment for EME

In this study, a prototype device for conductive vial EME from Extraction Technologies Norway (ETN) was used to perform the EME experiments. The equipment is depicted in Figure S2 [10]. Conductive vials (ETN) were used to hold the sample solution and the acceptor. The total inner vial volume was 600 μL. The sample vial and the acceptor

vial were connected by a support membrane union (ETN), containing a circular support membrane of porous polypropylene (ACCUREL PP2E, 100 μm thickness; Membrana Gmbh). The EME unit (sample vial + acceptor vial + support membrane union) was placed in a 10-position vial holder (ETN). The latter enabled simultaneous extraction of up to ten samples. The 10-position vial holder contained contact electrodes, and these coupled the electrical field through the conductive vials. The 10-position vial holder was mounted on an agitation system (Model MX-M; DLAB Scientific). Voltage was supplied by a model ES 0300-0.45 power supply from Delta Elektronika BV. A Fluke 287 multimeter was used to monitor the extraction current.

2.4 | Electromembrane extraction procedure

EME was performed according to the following procedure: First, 250 μL sample solution (pH ≈ 2.5) and 250 μL acceptor solution (100 mM HCOOH) were pipetted into the sample vial and acceptor vial, respectively. Second, 10 μL of NPOE or 2-undecanone was pipetted onto the support membrane. Third, the sample and acceptor vials were connected by the support membrane union to complete the assembly of the EME unit. Fourth, the EME unit was placed in the 10-position vial holder. The positive electrode was coupled to the sample vial, and the negative electrode was coupled to the acceptor vial. Extraction was performed for 30 min by simultaneous application of agitation at 800 rpm and voltage. The extraction current was monitored with an 8 Hz acquisition rate. After extraction, the EME unit was unassembled, and the acceptor was collected immediately and analyzed by ultra-high-performance LC-ultraviolet (UHPLC-UV) detection or UHPLC-MS/MS.

2.5 | UHPLC-UV detection

UHPLC-UV analysis was performed with a Dionex UltiMate 3000 RS UHPLC system (Thermo Fisher Scientific). Detection of 10 basic pharmaceutical substances was accomplished at 210 nm by a VWD-3400 UV/VIS detector (Dionex Corporation). An Eclipse Plus C18 RRHD column (50 × 2.1 mm, 1.8 μm; Agilent Technologies) was used for the separation (Figure S3). The mobile phase A consisted of 5:95 v/v acetonitrile and MQ-water with 0.1% formic acid, and mobile phase B consisted of 95:5 v/v acetonitrile and MQ-water with 0.1% formic acid. Gradient elution was performed as reported elsewhere [11]. The total run time was 10.0 min, the column temperature was 40°C, and the injection volume was set to 10 μL.

2.6 | UHPLC-mass spectrometry

Recovery and precision were established for 90 basic pharmaceuticals with the final generic methods B1 and B2, using UHPLC−MS/MS. UHPLC−MS/MS analysis was performed with an Agilent 1290 Infinity II UHPLC system (Agilent Technologies), consisting of a binary pump, an autosampler, and a column compartment with controllable temperature. An Eclipse Plus C18 column (50 × 2.1 mm, 1.8 μm; Agilent Technologies) was used for separation. The column temperature was 40°C and the injection volume was 1.0 μL. Mass spectrometric detection was performed with a model 6495 LC/TQ (Agilent Technologies) with positive electrospray ionization at 3 kV and with a desolvation gas temperature of 200°C. The system was operated in the dynamic multiple reaction monitoring mode, with a cycle time of 300 ms, resulting in a minimum dwell time of 4.52 ms. The mobile phase composition and the gradient were the same as with UHPLC-UV. Further details about UHPLC-MS/MS conditions are found in Table S1 and elsewhere [11].

2.7 | Calculations

Recovery (R) was calculated according to the following equation for each analyte:

$$\mathrm{R} = \frac{n_{a,final}}{n_{s,initial}} \cdot 100\% = \frac{V_a}{V_s} \cdot \frac{C_{a,final}}{C_{s,initial}} \cdot 100\% \qquad (1)$$

Here, $n_{\mathrm{s,initial}}$ and $n_{\mathrm{a,final}}$ are the number of moles of analyte originally present in the sample and the number of moles of analyte detected in the acceptor solution at the end of extraction, respectively. Correspondingly, $C_{s,initial}$ is the original concentration of analyte in the sample, and $C_{a,final}$ is the final concentration of analyte in the acceptor. V_{a} and V_{s} are the volumes of the acceptor and sample solution, respectively. Recoveries exceeding 85 % were defined as exhaustive extraction.

The matrix effect (ME, %) was calculated according to the following equation.

$$\mathrm{ME} = \frac{A_{post\ extraction\ spiked\ matrix}}{A_{unextracted\ neat\ standard}} \times 100\% \qquad (2)$$

$A_{\mathrm{unextracted\ neat\ standard}}$ is the peak area of analyte in a neat standard solution of equal concentration to post-extraction spiked acceptor. $A_{\mathrm{post\text{-}extraction\ spiked\ matrix}}$ is the peak area of the analyte in a post-extraction spiked acceptor, after the extraction of a blank matrix sample.

Repeatability was determined by calculating the relative standard deviation of the peak areas from

experiments performed in triplicate. The acceptance criterion for repeatability was set to ≤15%.

3 | RESULTS AND DISCUSSION

The present study reports experiments to optimize the sample solution, the acceptor phase, and the key operational parameters to complete the development of generic methods B1 and B2. A selection of ten basic pharmaceuticals, within a polarity range of $1.0 < \log P < 6.5$, were chosen as model analytes (Figure S1). These include metoprolol, pethidine, oxprenolol, noscapine, droperidol, promazine, hydroxyzine, chlorpromazine, thioridazine, and meclizine. All extractions were conducted from pooled human plasma samples, and acceptors were analyzed by UHPLC with ultraviolet detection (Figure S2). Primary experimental data encompassed recoveries, precision, and extraction current. The optimization process aimed to achieve exhaustive extraction, while maintaining a precision level within 15% relative standard deviation (RSD). Recoveries surpassing 85% were considered as exhaustive extraction [11]. Furthermore, based on previous experiences the upper limit for the extraction current was set at 50 μA [11].

3.1 | Optimization of the acceptor

The acceptor pH and composition are highly important for several reasons. First, in the context of extracting a basic analyte, maintaining an appropriately low pH in the acceptor phase is imperative to ensure that the analyte remains protonated. This protonation serves the critical purpose of entrapping the analyte within the acceptor, thereby effectively preventing its re-entry into the liquid membrane. Secondly, the acceptor must exhibit adequate acidity or possess buffer capacity to counteract any pH fluctuations arising from electrolysis during the extraction process. Given that EME involves the positioning of the cathode within the acceptor and the anode within the sample, the following electrolysis reactions occur:

$$\text{Acceptor}: \quad H_2O + e^- \rightarrow OH^- + \frac{1}{2}H_2 \tag{3}$$

$$\text{Sample}: \quad H_2O \rightarrow 2H^+ + \frac{1}{2}O_2 + 2e^- \tag{4}$$

If the acceptor is not sufficiently acidified or buffered, the pH in the acceptor may increase during extraction. Third, in the acceptor close to the liquid membrane, pH may be higher than in the bulk solution. If the acceptor is not sufficiently acidic in this boundary layer, the transfer of basic analytes into the bulk acceptor may be reduced.

In a preliminary series of experiments, a diverse array of acceptor solutions was tested (Tables S2 and S3). The acceptor solutions included diluted hydrochloric acid, phosphoric acid, trifluoroacetic acid, formic acid, acetic acid, and hydrogen phosphate: citric acid buffer solution. The latter was tested due to its buffering ability across a wide pH range (2.2–8.0). Each type of acceptor was systematically tested across three concentrations: 10, 25, and 100 mM, respectively, both with NPOE and 2-undecanone. The most important data are summarized in Figure 1.

With NPOE as the chosen liquid membrane (Figure 1A), the recoveries achieved for promazine, hydroxyzine, and chlorpromazine were very similar to all the tested acceptors. These model analytes are in the polarity range $3.4 < \log P < 4.5$ in the center of the extraction window of NPOE. In this part of the extraction window, the flux and the mass transfer in and out of the liquid membrane (T_1 and T_2, respectively) are well balanced, and excessive trapping in the liquid membrane is avoided. Due to favorable T_1 and T_2 conditions, these model analytes were not sensitive to the choice of acceptor.

Oxprenolol, pethidine, noscapine, and droperidol showed a different behavior and were more sensitive to the choice of acceptor. Among the acceptor solutions examined, it was evident that the stronger hydrochloric and trifluoroacetic acids, along with a mixed buffer system comprising hydrogen phosphate and citric acid, consistently yielded the highest recoveries for these model analytes. Pethidine, noscapine, and droperidol are within the range $2.5 < \log P < 3.0$, and are in the lower part of the extraction window. Oxprenolol is even more polar ($\log P = 2.2$) and is close to the lower limit of the extraction window. Most probably, these model analytes were affected by the boundary layer effect described above. Upon EME of basic substances, pH in the acceptor is elevated in the boundary layer. This boundary layer reduced the mass transfer into the acceptor (T_2) for noscapine and droperidol with relatively low pK_a-values (6.16 and 6.75, respectively). The boundary layer effect was strongest with the weak acid acceptors, while it was suppressed with the stronger hydrochloric and trifluoroacetic acids. The mixed buffer of hydrogen phosphate and citric acid provided a similar performance as the strong acids. Citric acid was enriched in the boundary layer, and due to the electrical field and the triprotic properties of citric acid, low pH was maintained in this region. The boundary layer effect also affected oxprenolol and pethidine. Although they are stronger bases ($pK_a = 9.27$ and 8.14, respectively), they are more polar and were prone to T_1-limitations. Even with a very weak boundary layer, the flux out of the membrane (T_2) was slightly reduced, and this in turn

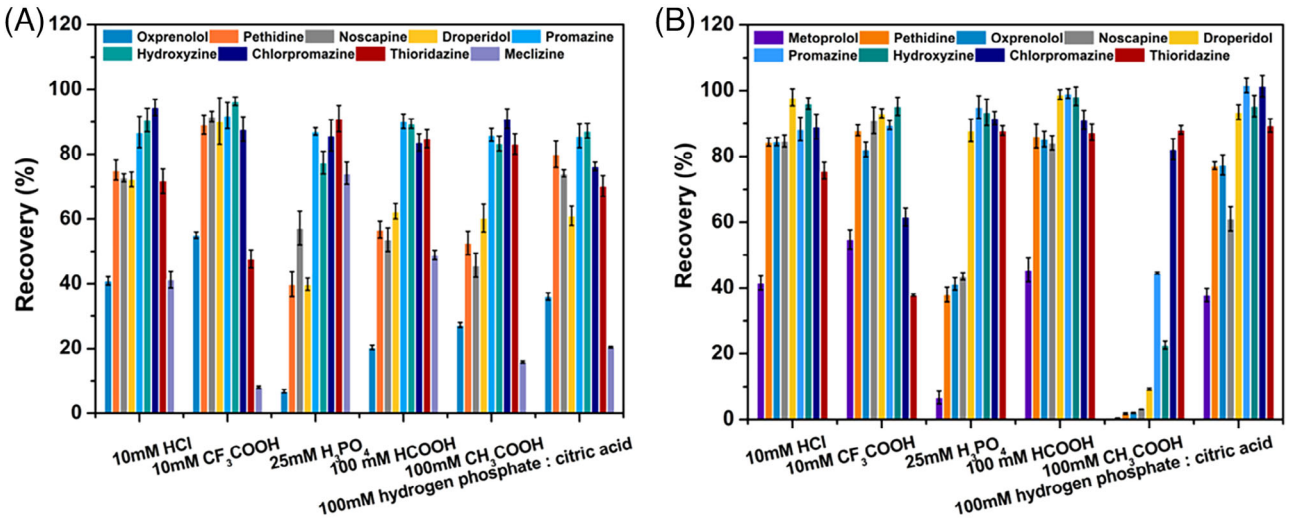

FIGURE 1 Recovery with different acceptors. (A) 2-nitrophenyl octyl ether (NPOE) and (B) 2-undecanone as liquid membrane. Extraction time: 30 min, extraction potential: 50 V.

decreased the flux of the T_1-limited model analytes into the liquid membrane.

The very non-polar model analyte thioridazine (log P = 5.47) was close to the upper limit of the extraction window, while meclizine (log P = 6.39) was outside the extraction window. Both model analytes were T_2-limited and showed significant variations using different acceptors. Apparently, phosphoric acid and formic acid were the most efficient acceptors for these model analytes.

Based on the above experiences, 100 mM formic acid (pH 2.5) was selected as the recommended acceptor for generic method B1. This acceptor can be injected directly into LC-MS, and can therefore be transferred from EME to an auto-sampler of a LC-MS system. An identical set of experiments was conducted with 2-undecanone as acceptor (Figure 1B). The results were similar to the data with NPOE, except that low recoveries for unknown reasons were obtained using diluted acetic acid. Since 100 mM formic acid provided high recoveries with 2-undecanone, this was selected as the acceptor also for generic method B2.

3.2 | Optimization of the sample diluent

The pH within the sample solution is less critical for two reasons. First, a crucial factor is the formation of a boundary layer in the sample solution at the liquid membrane interface, characterized by a lower pH than the bulk sample solution [12]. This local pH reduction is particularly advantageous for facilitating mass transfer processes. In scenarios where the pH within the bulk sample solution fails to reach an adequately low threshold, basic analytes may experience protonation upon encountering the boundary layer, effectively compensating for any suboptimal pH conditions. Secondly, basic analytes can transfer the liquid membrane even in their neutral form. While this mode of extraction may entail a decrease in extraction kinetics, it remains a viable pathway for analyte extraction.

Consequently, a basic analyte can be extracted directly from the original sample, provided the compound is in a fully protonated state. To fulfill this criterion, it is imperative that the pH within the sample solution is maintained at least 2–3 units below the pKa value corresponding to the specific basic analyte in question. In cases where this condition is not met or if the pH levels exhibit variability across different samples, the inclusion of a suitable sample diluent becomes imperative.

In a new set of experiments, different sample diluents were tested. Pooled plasma was chosen as the sample, and the sample volume was set to 125 μL. Various aqueous dilutions of phosphoric acid, trifluoroacetic acid, hydrochloric acid, and formic acid were tested. The volume of these diluents was set to 125 μL. To accommodate the buffer capacity inherent to plasma, the molarity of hydrochloric and trifluoroacetic acid was adjusted to 180 mM, achieving a final sample solution pH of approximately 2.5. Conversely, for phosphoric and formic acid, the molarities of the sample diluents were set at 250 mM and 2 M, respectively, to achieve the desired pH level of 2.5 within the final sample solution.

The recoveries using various sample diluents are summarized in Figure 2A, employing NPOE as the liquid membrane and 100 mM formic acid as the acceptor. For oxprenolol, pethidine, and noscapine (all featuring log P

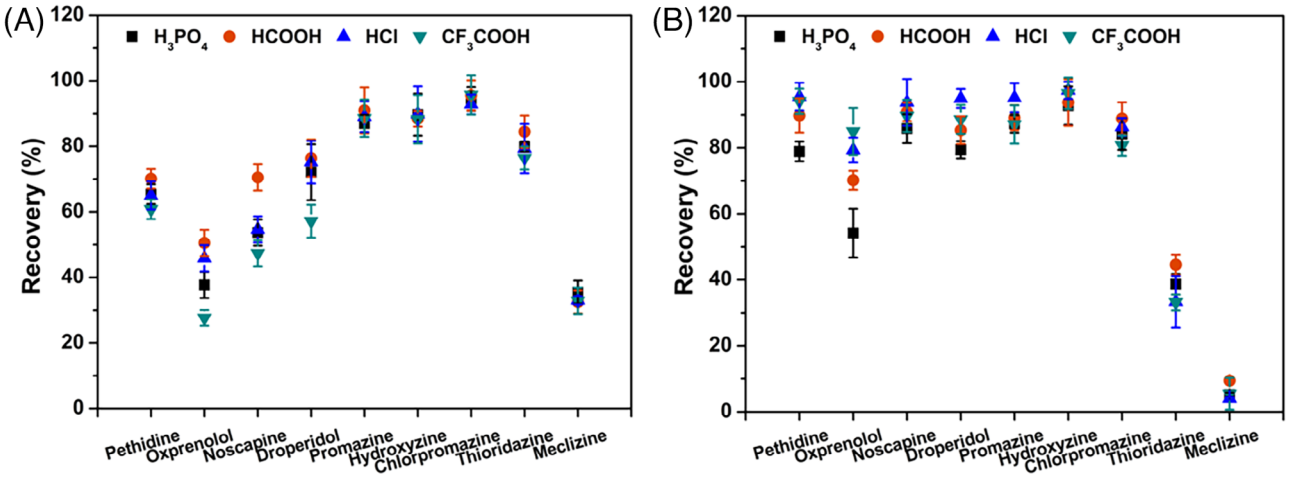

FIGURE 2 Recovery with different sample solutions using 2-nitrophenyl octyl ether (NPOE) as a liquid membrane. (A) 100 mM formic acid and (B) 10 mM TFA as acceptor. Extraction time: 30 min, extraction potential: 50 V.

values below 2.6), the most favorable recoveries were consistently achieved when formic acid was utilized as the sample diluent. In contrast, recoveries for analytes exhibiting lower polarity (log $P > 2.6$) exhibited no significant sensitivity to the choice of sample diluent. This consistent trend was confirmed using 10 mM trifluoroacetic acid as the acceptor, as illustrated in Figure 2B, alongside parallel experiments employing 2-undecanone as the liquid membrane (data not displayed). Consequently, formic acid emerged as a clear choice for the recommended sample diluent, demonstrating its suitability for both B1 and B2 methods.

In the next series of experiments, 125 μL of pooled plasma was meticulously blended with an equal volume of either 0.25, 0.50, 1.0, or 2.0 M HCOOH, respectively (Figure S4). With 0.25 M HCOOH, the pH within the final sample solution was around 4.0, whereas 2.0 M HCOOH yielded a pH value of approximately 2.5. Employing NPOE as the liquid membrane, it was observed that recoveries remained largely unaltered within the pH range of 2.5–4.0. In the case of 2-undecanone, however, distinctive patterns emerged. For analytes characterized by log P values exceeding 3.0, sensitivity to pH within the sample solution within the range of 2.5–4.0 was minimal. Conversely, for model analytes of higher polarity (log $P < 3.0$), recoveries increased at elevated concentrations of formic acid. This phenomenon suggests a potential enhancement in ion pairing with formic acid, which subsequently amplified the efficiency of T_1 for model analytes situated close to the lower limit of the extraction window. Based on these experiences, 0.5 M formic acid was selected as the sample diluent for generic method B1, while 1.0 M formic acid was designated for B2.

3.3 | Optimization of the extraction potential

Generally, recoveries increase with increasing extraction potential up to a certain voltage (V_{max}) [4]. Above V_{max}, the mass transfer is no longer limited by the strength of the electrical field, but rather by the convectional conditions and the mass transfer within the sample solution. At V_{max} and above, the EME system tends to be less stable, due to elevated extraction current and excessive electrolysis. Therefore, EME is often performed below V_{max}. In the pursuit of optimizing extraction potential, a comprehensive series of experiments was devised, and the results are summarized in Figure 3. With NPOE, recoveries increased with voltage up to V_{max} at 150 V (Figure 3A). This behavior was in agreement with the literature. Notably, the corresponding plots of extraction current (Figure 3B) unveiled that the EME system exhibited remarkable stability at 100 V. However, at the critical threshold of 150 V (V_{max}), the extraction current suddenly increased at the 14-minute mark, accompanied by a subsequent destabilization of the extraction system. Consequently, 100 V was selected as the recommended extraction potential with generic method B1.

The current was higher with 2-undecanone as the liquid membrane because this solvent is less hydrophobic than NPOE. The optimization with 2-undecanone spanned the voltage range of 0 to 75 V. As shown in Figure 3C, recoveries increased with increasing voltage up to V_{max} at 60 V. However, the current increased significantly above 50 V, and for system stability reasons, 50 V was selected as the final extraction potential in generic method B2 (Figure 3D).

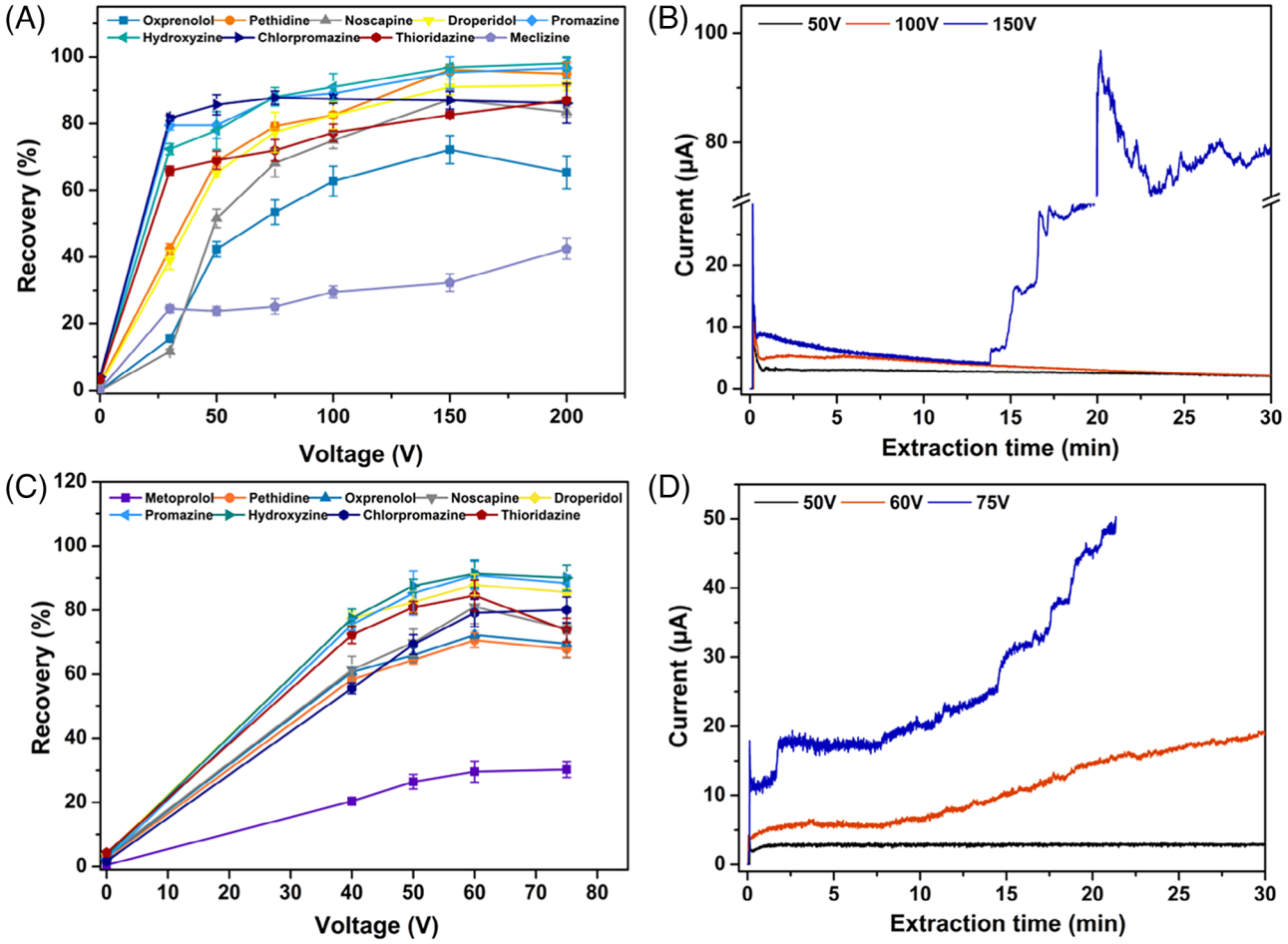

FIGURE 3 Recovery and extraction current (the average current for three simultaneous extraction replicates) with different extraction potentials. (A, B) 2-nitrophenyl octyl ether (NPOE) and (C, D) 2-undecanone as a liquid membrane. Extraction time: 30 min.

3.4 | Optimization of the extraction time

Generally, recoveries increase with increasing extraction time until a certain time point, where the extraction system enters steady-state conditions [4]. At this time point (t_{max}), there is no further gain by extending the extraction time, and in some cases, recoveries even start decreasing again due to excessive electrolysis and drifting pH. To comprehensively explore the impact of extraction time, a series of optimization experiments were conducted and their findings are summarized in Figure 4. With NPOE as the liquid membrane, the recoveries displayed the anticipated trend until reaching t_{max} at 30–40 min (Figure 4A). The initial extraction rates exhibited a distinct correlation with the polarity of the model analytes and their positioning within the extraction window of the liquid membrane. Notably, the highly hydrophobic compound, meclizine, exhibited a slower initial extraction rate, owing to its location outside the extraction window of NPOE. The initial extraction rates were higher for oxprenolol, noscapine, and pethidine (all compounds with log $P < 2.6$), but the model analytes with log $P > 2.6$ were extracted even faster. The latter group of model analytes is in the center of the extraction window for NPOE. Based on this experiment, 30 min was selected as the recommended extraction time for generic method B1.

Figure 4B presents the kinetic data obtained with 2-undecanone. This liquid membrane exhibited a slightly adjusted extraction window, favoring more polar analytes. Remarkably, metoprolol (log $P = 1.76$) demonstrated reasonable extraction efficiency with this liquid membrane. On the other hand, meclizine (log $P = 6.6$) was not extracted in B2 due to trapping in the liquid membrane. Also, for generic method B2, 30 min was selected as the final extraction time.

The experiments reported in Figure 4 were obtained with 250 μL sample solution (125 μL plasma and 125 μL sample diluent), using the first-generation conductive EME vials with a total volume of 600 μL. In parallel, we explored new prototype vials with a total volume of 300 μL, featuring a 100 μL sample solution (comprising plasma + diluent). Recoveries as a function of time are presented

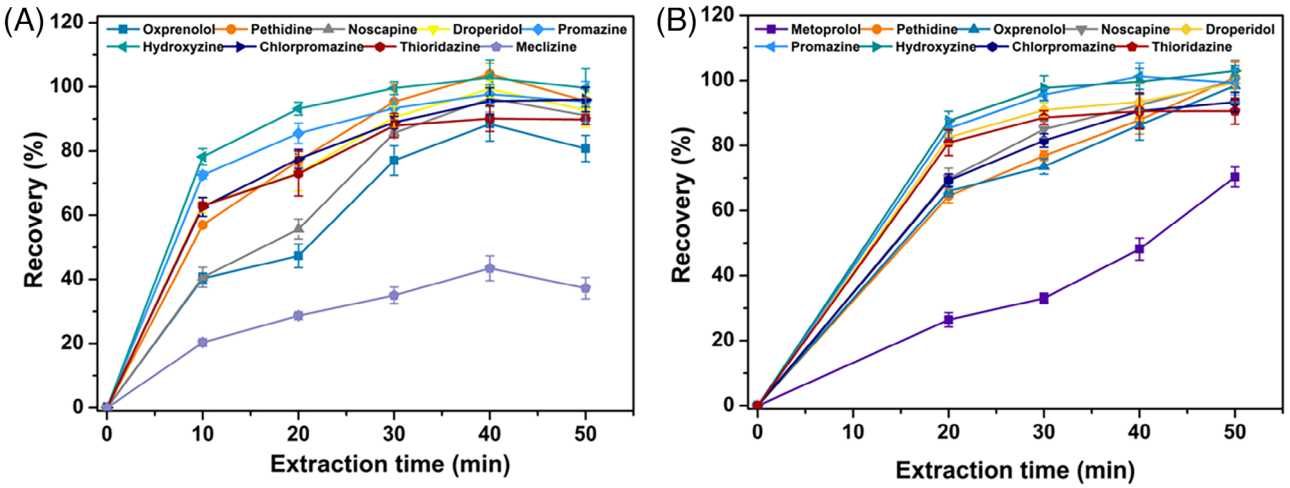

FIGURE 4 Recovery versus extraction time with 600 μL vials. (A) 2-nitrophenyl octyl ether (NPOE), 100 V and (B) 2-undecanone, 50 V. Sample volume: 250 μL.

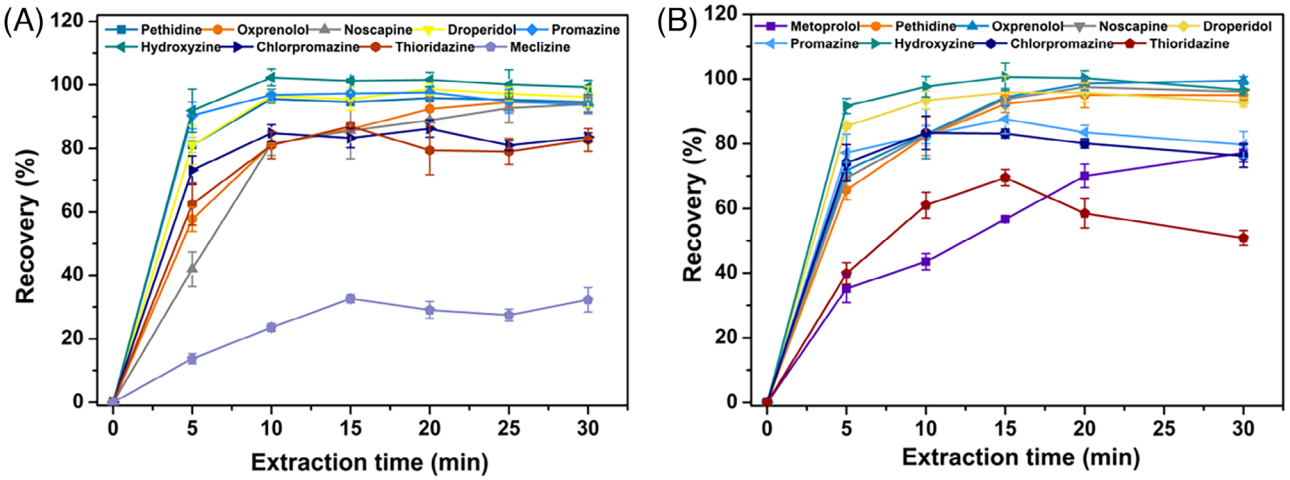

FIGURE 5 Recovery versus extraction time with 300 μL vials. (A) 2-nitrophenyl octyl ether (NPOE), 100 V and (B) 2-undecanone, 50 V. Sample volume: 100 μL.

in Figure 5A,B, encompassing data obtained with NPOE and 2-undecanone, respectively. In comparison to Figure 4, it becomes apparent that extraction kinetics were notably accelerated with the smaller vials, and t_{max} was reduced to 5–10 min. This observation supported that extraction at V_{max} is limited by the mass transfer in the bulk sample. Therefore, 10 min was selected as the recommended extraction time for both generic methods, when performed using small vials.

3.5 | Extraction performance under optimized conditions

Generic methods B1 and B2 are summarized in Table 1. Both methods represent green sample preparation, as the consumption per sample is limited to ten microliters of organic solvent and less than 400 microliters of dilute formic acid. In a final set of experiments, extraction recovery, precision, and potential LC-MS MEs with B1 and B2 were investigated. As seen in Table 2, within the extraction window of B1, all model analytes except oxprenolol were extracted exhaustively with recoveries between 87 and 100 %. Oxprenolol (log P = 2.17) was extracted with a recovery of 77%. With the exception of metoprolol, all model analytes within the extraction window of B2 reached exhaustive extraction with recoveries in the range between 86% and 98%. For metoprolol (log P = 1.76), the recovery was 48 %. All data for intra-day precision were within 2.2% and 9.7% RSD and were considered acceptable.

In the next set of experiments, 90 different basic pharmaceuticals were extracted to confirm the extraction

TABLE 1 Summary of experimental conditions for generic methods B1 and B2.

	B1	B2
Applicability range	Mono- and di-bases $2.0 < \log P < 6.0$ Exhaustive extraction	Mono-bases $1.0 < \log P < 4.5$ Selective extraction
Experimental conditions		
Liquid membrane	2-nitrophenyl octyl ether, 10 μL	2-undecanone, 10 μL
Sample	Plasma	Plasma
Sample diluent	0.50 M formic acid	1.0 M formic acid
Sample solution	125 μL sample + 125 μL sample diluent	125 μL sample + 125 μL sample diluent
Acceptor	250 μL 100 mM HCOOH	250 μL 100 mM HCOOH
Extraction potential	100 V	50 V
Agitation	800 rpm	800 rpm
Extraction time	30 min	30 min

TABLE 2 The recoveries, relative standard deviations (RSDs), and matrix effects (ME) of the basic drug spiked plasma samples.

	log	NPOE ($n = 3$)			2-undecanone ($n = 3$)		
Analyte	***P***	**Recovery (%)**	**RSD (%)**	**ME (%)**	**Recovery (%)**	**RSD (%)**	**ME (%)**
Metoprolol	1.76	18	5.3	91	48	2.3	91
Pethidine	2.46	95	7.2	96	88	6.7	97
Oxprenolol	2.17	77	5.2	94	86	3.4	97
Noscapine	2.58	87	2.2	96	92	2.8	96
Droperidol	3.01	91	7.7	99	93	3.7	93
Promazine	3.93	93	3.4	101	95	5.9	99
Hydroxyzine	3.41	100	2.8	97	98	3.4	94
Chlorpromazine	4.54	89	9.7	104	91	6.5	109
Thioridazine	5.47	88	4.7	95	81	5.5	98

Abbreviations: ME, matrix effect; NPOE, 2-nitrophenyl octyl ether; RSD, relative standard deviation.

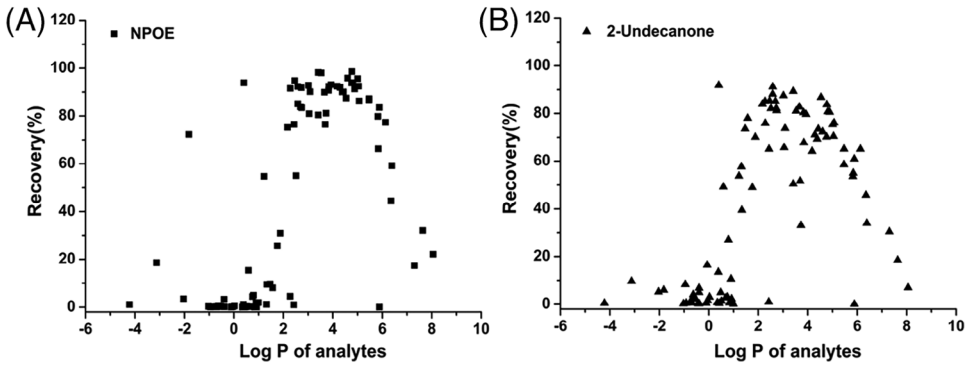

FIGURE 6 Recoveries versus log *P* for 90 model bases using (A) 2-nitrophenyl octyl ether (NPOE) and (B) 2-undecanone as liquid membrane, under optimized extraction conditions (Table 1) from human plasma samples. Each point represents the average recovery of triplicate extraction.

window of B1 and B2 under optimized conditions. As seen in Figure 6A, the majority of compounds in the range $2.0 < \log P < 6.0$ were extracted exhaustively from plasma with generic method B1. Figure 6B shows a similar plot for B2. This method provided a higher level of selectivity, and recoveries varied more from compound to compound within the extraction window. While NPOE in generic method B1 extracted the analytes based on cation-π

interactions and hydrogen bond interactions, 2-undecanone in B2 was limited to hydrogen bond interactions. For this reason, generic method B2 provided somewhat more selectivity.

In addition, extraction of the model analytes with B1 and B2 from human plasma, followed by UHPLC-MS/MS analysis, was checked for potential MEs. ME values obtained with B1 and B2 were all in the range of 91%–104% and 90%–109%, respectively (Table 2). Thus, no ion suppression or enhancement was observed with the proposed generic methods.

4 | CONCLUDING REMARKS

In this work, generic methods have been developed for EME of mono- and dibasic analytes (z = +1 and +2) with log $P > 1.0$. In combination with commercially available EME equipment, EME can now be conducted under standardized conditions. This is highly important for the acceptance of EME by the scientific community and for implementation in routine analytical chemistry. Work is in progress to develop generic methods also for polar bases and for acids, and by such develop a complete roadmap for EME. Generic methods can be used directly, or they can serve as a starting point for further optimization.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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