Vortex Assisted Liquid-Liquid Microextraction with Back Extraction of Repaglinide, Glibenclamide and Glimepiride in Water Samples

(Mikro Pengekstrakan Berbalik Cecair-Cecair Berbantu Vorteks bagi Repaglinida, Glibenclamida dan Glimepirida dalam Sampel Air)

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ABSTRACT

A new analytical method based on vortex-assisted liquid-liquid microextraction with back extraction (VALLME-BE) coupled with high performance liquid chromatography was developed for the simultaneous determination of antidiabetic drugs; repaglinide, glibenclamide, and glimepiride in water samples. Chromatographic separation was achieved using C18 column (250 × 4.6 mm × 5 µm) and methanol-phosphate buffer (pH3.7) in the ratio of 70:30 v/v as a mobile phase at a flow rate of 1 mLmin^{-1}. VALLME-BE was performed using 200 µL of n-octane dispersed into the aqueous sample (10 mL) with the aid of vortexing agitation. Then, the analytes were back-extracted from the organic solvent to 0.05 M NaOH (40 µL). Under these conditions, enrichment factor of 155-fold was achieved. The developed VALLME-BE method showed excellent linearity in the range of 30 to 1000 µgL^{-1} with limit of detection (LOD) of 0.41-1.66 µgL^{-1} and limit of quantification (LOQ) of 1.38-5.54. 41-1.66 µgL^{-1}. VALLME-BE was applied for the determination of repaglinide, glibenclamide and glimepiride in water samples with the recoveries ranged from 83-109%. The relative standard deviation for inter-day and intra-day precision was less than 9.9%.

Keywords: Glibenclamide; glimepiride; HPLC-UV; repaglinide and vortex assisted liquid-liquid microextraction with back extraction

ABSTRAK

Suatu kaedah analitikal yang baharu berdasarkan pengekstrakan berbalik - mikro pengekstrakan cecair-cecair berbantu vorteks (VALLME-BE) digandingkan dengan kromatografi cecair berprestasi tinggi telah dibangunkan untuk penentuan serentak ubat anti-diabetik; repaglinida, glibenklamida dan glimepirida di dalam sampel air. Pemisahan kromatografi telah dicapai menggunakan turus C18 (250 × 4.6 mm × 5 µm) dan penimbal methanol-fosfat (pH3.7) dengan nisbah 70:30 v/v sebagai fasa bergerak pada kadar aliran 1 mLmin^{-1}. VALLME-BE telah dilakukan dengan menggunakan 200 µL n-oktana yang disebarkan ke dalam sampel akues (10 mL) dengan bantuan pengadukan. Kemudian, pengekstrakan berbalik dilakukan terhadap analit daripada pelarut organik kepada 0.05 M NaOH (40 µL). Di bawah keadaan optimum, faktor pengayaan sebanyak 155-lipat telah dicapai. Kaedah VALLME-BE yang dibangunkan telah menunjukkan kelinearan yang baik dalam julat 30 hingga 1000 µgL^{-1} dengan had pengesanan (LOD) sebanyak 0.41-1.66 µgL^{-1} dan had pengkuantitian (LOQ) sebanyak 1.38-5.54 µgL^{-1}. VALLME-BE digunakan untuk pengekstrakan repaglinida, glibenklamida dan glimepirida dengan julat pengembalian semula adalah 83-109%. Sisihan piawai relatif untuk inter-hari and intra-hari mempunyai kepersisan kurang daripada 9.9%.

Kata kunci: Glibenklamida; glimepirida; HPLC-UV; repaglinida dan pengekstrakan berbalik - mikro pengekstrakan cecair-cecair berbantu vorteks

INTRODUCTION

Diabetes mellitus is one of common diseases affecting human health with 150 million people around the world are estimated sufferings from it. This is a serious public health problem, as incidence and prevalence rates are increasing worldwide (Forouhi & Wareham 2014). Among diabetes patients, ~ 90% of them are suffered with

diabetes type II (non-insulin-dependent) (Siddiqui 2014). Repaglinide, glibenclamide and glimepiride are well known compounds that clinically used in the treatment of type II diabetes mellitus (T2D) (Bojarska et al. 2019; El-Zaher et al. 2016). The hyperglycemia of T2D is due to inappropriately low insulin levels caused by a combination of defects in insulin secretion and action. Repaglinide is an insulin secretagogue with early onset and short duration of action that mainly targets postprandial hyperglycemia. Meanwhile, glimepiride and glibenclamide are the potent second generation oral sulfonylurea antihyperglycemic agents that increase insulin release from pancreatic beta cells (Fachi et al. 2016; Gumieniczek & Berecka 2016).

As antidiabetic drugs are extensively used for the diabetes treatment; thus, they are continuously being released to the environment through wastewater discharges from wastewater treatment plants. The presence of pharmaceutical residues in the aquatic environment has been recognized as one of the most urgent emerging environmental issues. Toxicity of glibenclamide has been proven to have harmful effects on humans and animal species such as DNA, mutagenesis, genotoxicity, and oxidative stress (Ibarra-Costilla et al. 2010). Although the effect of repaglinide and glimepiride on environmental water still not clear, it is still important to analyse them as it is widely produced and consumed around the world.

In the last decade, several analytical methods have been developed to investigate the occurrence of the pharmaceutical compounds approved for human usage in the aquatic environment (Radke 2010; Selahle & Nomngongo 2020). Several analytical methods have been developed for the determination of pharmaceuticals such as beta-blockers, antibiotics, anti-inflammatory drugs, antidepressants, and lipid regulators (Al-odaini et al. 2010; Kasprzyk-Hordern et al. 2007; Nannou et al. 2015). However, the methods for the analysis of antidiabetic drugs in environmental waters are scarce as the developed methods are restricted to their individual determination in biological matrices (Chen et al. 2019; Mokhtar et al. 2020; Omran et al. 2019) or in pharmaceutical preparations. At present, analytical methods reported for the determination of repaglinide, glibenclamide, and glimepiride in the aquatic environment are limited. In 2010, Lopez-Serna et al. developed online-solid phase extraction-liquid chromatography-electrospray-tandem mass spectrometry for determination of 74 pharmaceutical compounds including glibenclamide in environmental and waste waters. Glibenclamide with other compounds was extracted using Oasis MCX from environmental water sample and determined using Q-Exactive Orbitrap

high resolution accurate mass spectrometry (Abdallah et al. 2019). Solid phase extraction (SPE) is widely used as sample preparation from water sample. However, SPE drawbacks are tedious and time-consuming involving multiple extraction steps. Loss of analytes can occur due to multi steps involving evaporation step prior to analysis (AbuRuz et al. 2005, 2003).

Vortex-assisted liquid-liquid microextraction with back-extraction (VALLME-BE) is a promising alternative technique to the traditional sample preparation as it is simple and cost efficient (Feng et al. 2017; Lian et al. 2014; Pizarro et al. 2014). The first step (VALLME) is the extraction of the analyte from a large amount of aqueous phase (donor phase) to small volume of organic phase (intermediate phase). In the second step, the target analyte was back-extracted from organic phase to a micro-volume of aqueous phase (acceptor phase) and this is referred as back extraction (BE). The extract can be conveniently sampled and injected directly into LC for analysis (Namieśnik et al. 2015).

Towards this end, of the plethora of microextraction techniques, the VALLME-BE seemed to be the best candidate. Thus, this paper is dedicated to the development of a VALLME-BE pretreatment method for the simultaneous determination of repaglinide, glyburide, and glimepiride in environmental water. To the best of our knowledge, the VALLME-BE method has not previously been applied for the simultaneous determination of repaglinide, glibenclamide, and glimepiride in the literature.

MATERIALS AND METHODS

CHEMICALS AND REAGENTS

Repaglinide, glibenclamide, and glimepiride were kindly donated by Hikma Pharmaceuticals (Amman, Jordan). Materials and reagents used were obtained from the following sources: HPLC-grade methanol (99.96%), phosphoric acid (85%, w/w), 1-octanol, ethyl acetate was purchased from Merck (Darmstadt, Germany). Toluene was from Fisher Scientific (Milwaukee, WI, USA); 1-heptanol (99.9%) and butyl acetate (99.0%) were from Fluka (Buchs, Switzerland); sodium hydroxide (98.0%), R&M Marketing (Essex, UK); n-octane (99%), Acrōs (Geel, Belgium). n-heptane (99.0%), 1-hexanol (99%), sodium phosphate monobasic monohydrate and sodium phosphate were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water (resistivity, 18.2 MΩcm) was produced by Millipore water purification system (Molsheim, France) and used throughout for the preparation of solutions.

HPLC CONDITIONS

Separation was performed using Shimadzu HPLC (SPD-20A) system equipped with UV Detector (LCD-20A) (Kyoto, Japan). The separations were carried out using Thermo-Fisher Hypersil Gold ODS C18 (250 × 4.6 mm × 5µm) (Thermo fisher Scientific Lnc., St. Wyman, Waltham, MA, USA). The mobile phase consisted of a mixture of methanol and phosphate buffer (15 mM, pH 3.7) with ratio of 70:30, v/v. The mobile phase was filtered through 0.22 µm Agilent Technologies nylon membrane filter (Waldbronn, Germany) and degassed for 20 min in an ultrasonic bath prior to its use. The injection volume and the flow rate were 10 µL and 1 mLmin^{-1}, respectively. The detection wavelength was set at 234 nm.

PREPARATION OF STOCK AND WORKING SOLUTION

Stock standard solutions (200 mgL^{-1}) of repaglinide, glibenclamide and glimepiride were prepared by dissolving the desired amounts in methanol and stored at 4 °C. Working standard solutions were prepared in 10 mL volumetric flask by appropriate dilution of stock solution.

EXTRACTION PROCEDURE FOR MICROEXTRACTION VORTEX ASSISTED LIQUID-LIQUID MICROEXTRACTION-BACK-EXTRACTION

The extraction procedure was performed in two separate steps. Initially, a microextraction vortex assisted liquid-liquid (VALLME) was performed, i.e. standard and sample solution (10 mL) pH was adjusted to 6.0 using NaOH solution (0.1 M). Then, the sample solution was transferred into a volumetric flask (10 mL) and top-up the mark. Next, the solution was spiked with n-octane (200 µL). The mixture was vortexed vigorously (2500 rpm) for 60 s to from fine droplets using a vortex agitator. The mixture was left for ~1 min to reform the n-octane single drop. The organic phase was collected and then transferred to centrifuged tube (1.5 µL) using micropipette. Thereafter, a back extraction (BE) procedure was performed by the addition of 40 µL NaOH solution (0.05 M). The mixture was again vortexed (1500 rpm) for 1 min then centrifuged (4000 rpm) for 1 min. Finally, 10 µL from the bottom aqueous drop was carefully withdrawn using a micro-syringe and directly injected into HPLC.

METHOD VALIDATION

The validation parameters of the proposed methods such as linearity, limits of detection (LOD), limits of quantitation (LOQ), repeatability, and recovery were tested after subjecting the working standards to the VALLME-BE procedure. Individual analyte was assessed for linearity using five-point calibration curve over the range 30 - 1000 µgL^{-1}. The calibration curve was plotted, and the slope and y-intercept of the curve was determined with linear regression.

REAL SAMPLE ANALYSIS

Six water samples (sea and river water) were collected at different places around Pulau Pinang, Malaysia to study the applicability of the proposed VALLME-BE procedure. After sampling, all samples were stored at 4 °C in the dark until use. Prior to the extraction, the water samples filtered through 0.45 µm membrane filters to remove impurities. Repeatability was investigated by injecting six replicates of three different concentrations levels of 30, 500, and 1000 µgL^{-1} and was expressed as relative standard deviation (%RSD). Accuracy was determined by evaluating the recovery of the analyte at low, medium and high concentration levels. The samples were spiked at three levels concentration (30, 500, and 1000 µgL^{-1}).

RESULTS AND DISCUSSION

OPTIMIZATION OF VALLME-BE PARAMETERS TYPE AND VOLUME OF THE INTERMEDIATE PHASE

The selection of solvent as intermediate phase (IP) should be based on its ability to form good emulsion after vortexing and possess a higher density as compared to water to reformed on the bottom of the tube which facilitate the transfer of such small extraction phase (Çabuk & Köktürk 2013). Seven extraction solvents including heptanol, hexanol, octanol, butyl acetate, octane, heptane, and hexane were tested for the extraction based on their polarities and interaction with analytes.

Figure 1(a) shows the effect of different types of solvents on the enrichment factor (EF) of repaglinide, glibenclamide, and glimepiride. Alcoholic solvents such as 1-hexanol, 1-heptanol and 1-octanol and ester, butyl acetate showed the lowest extraction efficiency among the selected solvents. This is due to the low affinity of analyte to these solvents which affected the mass transfer of analyte from donor phase into intermediate phase (Ho et al. 2004). Amongst n-alkane groups, octane exhibited the highest extraction efficiency due to its high solvation factor and high back extraction efficiency to acceptor phase. The analyte was also a lipophilic and non-polar compound. Hence, its interaction with non-

polar solvent gives the highest EF. Another factor is the volume of reformed layer, because octane reformed a higher layer volume than heptanol and hexane according to their polarity. Therefore, octane was selected for further optimization studies.
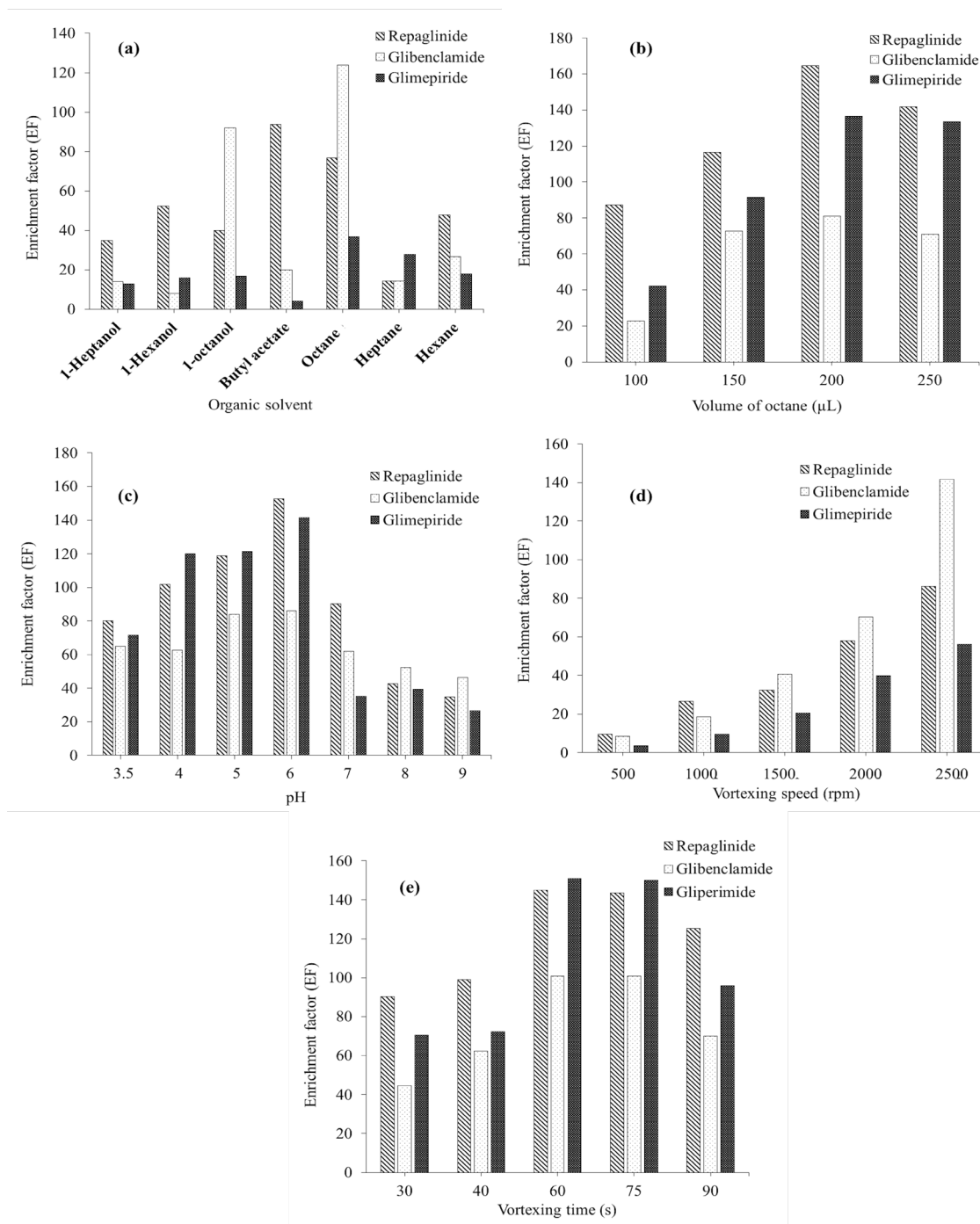

FIGURE 1. Effect of (a) extraction solvent (b) volume of extraction solvent (c) pH (d) vortexing speed and (e) vortexing time on the enrichment factor of repaglinide, glibenclamide and glimepiride. MSPE conditions: repaglinide, glibenclamide and glimepiride concentration: 1 mgL^{-1}, desorption eluent: NaOH 0.05 M, volume of eluent: 40 mL, vortex at 1500 rpm for 60 s

The volume of extraction solvent influences the ratio of analyte between donor phase/intermediate phase (DP/IP) and intermediate phase/acceptor phase (IP/AP) as it affects the equilibrium and the transfer rates of the analyte between both phases. Furthermore, the selection of suitable ratio was very important because it improves the partitioning of the analyte between the two immiscible phases (Makahleh et al. 2015). In this study, different volumes of n-octane in the range from 100 - 250 µL were used. Based on the result depicted in Figure 1(b), the EF increased as the volume of n-octane increased up to 250 µL and dropped thereafter. This might be due to the dilution factor which reduced the mass transfer of analyte in intermediate phase to the acceptor phase. Therefore, 200 µL of octane was selected for further analysis.

pH OF DONOR PHASE

The suitable pH of donor phase is a key factor to ensure analytes are transfer into organic solvent (intermediate phase). As depicted in Figure 1(c), the pH range was studied from pH 3.50 to 9. Based on the data obtained, the enrichment factor increased as the pH increased from 4 to 5 and dropped after pH 6.

Two of the analytes are basic (glibenclamide and glimepiride) and the expected pH that suppress the ionization should be higher than the pK_a, so it should be higher than 5. Meanwhile, repaglinide contains both acidic and basic functional group which make the predication of the pH not easy. According to the moderately high molecule size of the analytes; the ionization of the basic and acidic functional groups (which affected by pH) could not be the major driving force for the extraction, instead the hydrophobicity (the log P of the analyte is relatively high) of the molecule could be the main driving force which could justify the non-rational results of the enrichment factor in pH study. Therefore, pH 6 was selected as optimum pH for subsequent analysis.

VORTEX SPEED AND TIME

A vortex agitator was used to swirl the two immiscible liquid phases and create vortex emulsification between both phases. Furthermore, vortexing the immiscible phase during the extraction and back-extraction is essential in order to form fine droplets. Thus, the contact surface area was increased and further enhanced the mass transfer of the analyte (Yiantzi et al. 2010). The vortex speed range

was studied from 1000 to maximum speed of 2500 rpm. It was observed that the extraction efficiency increased with the increased of the vortexing speed. The enhancement in the emulsification process broke down the single drop into smaller droplets has resulted in the increasing of the specific surface area and the mass transfer of repaglinide, glibenclamide, and glimepiride. As shown in Figure 1(d), the highest extraction efficiency achieved at the maximum vortexing speed at 2500 rpm. Hence, that maximum speed was chosen for the subsequent study.

Vortexing time is another important parameter which influences the emulsification process and affects the equilibrium between the donor phase and intermediate phase and also the mass transfer of the analyte. Different durations from 30 to 120 s of vortex agitation were studied in VALLME procedure. Based on the results in Figure 1(e), it was found that the extraction efficiency was improved as the analyte was extracted from aqueous phase into organic phase and whenever vortexing time increased, the analyte was back extracted into donor phase. Therefore, 60 s was selected in vortexing time for further study.

ACCEPTOR PHASE CONCENTRATION AND VOLUME

In the second step, the anti-diabetic drugs were back-extracted from the octane into the NaOH as the acceptor phase (aqueous phase, AP). The analytes should be in the ionized form in the acceptor phase to avoid the back-extraction in octane (Alshishani et al. 2016). Repaglinide, glibenclamide, and glimepiride is a basic compound attributed to an amine group. They are existing predominantly in an ionized form at lower pH. Therefore, in order to find suitable pH medium, sodium hydroxide (NaOH) was selected as acceptor phase. The effect of NaOH concentration was studied in the range of 0.025 - 0.100 M. Referring to Figure 2(a), the extraction efficiency was increased as the concentration of NaOH increased and the concentration of 0.05 M NaOH gave the highest extraction efficiency. Hence, 0.05 M NaOH was selected for further studies. The concentration of NaOH above 0.1 M was neglected due to the corrosion effect on the injector and other parts of HPLC (Makahleh et al. 2015).

The volume of acceptor phase should also be carefully selected in order to find the optimum volume for high recovery and avoid to the dilution of target analytes. The volume is directly associated with mass transfer of target analytes from the intermediate phase to the accepter phase. The range effect of volume (15 - 40 µL) of accepter phase was investigated. The EF was presented

in Figure 2(b). The volume less than 20 µL were not considered as it is insufficient for the HPLC analysis. The enrichment factor increased when the volume of NaOH increased until 40 µL then dropped thereafter. The increment may be due to sufficient volume of NaOH to extract the targeted analytes in the intermediate phases.
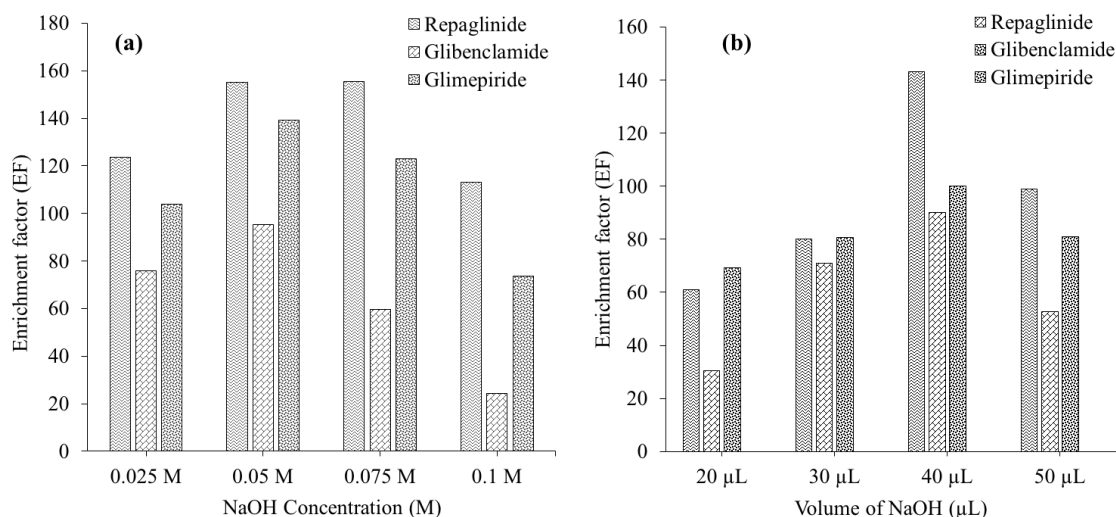

FIGURE 2. Effect of (a) NaOH concentration and (b) NaOH volume, on enrichment factor of repaglinide, glibenclamide, and glimepiride. Experimental conditions, adsorption: Repaglinide, glibenclamide and glimepiride concentration; 1 mgL⁻¹ adjusted to pH 6, volume of octane; 200 µL, the pH 6, vortexing speed; 2500 rpm for 1 min. Desorption conditions: vortex at 1500 rpm for 60 s

The decreased in EF at 50 µL could be justified by decrease of drugs final concentration in the NaOH phase due to dilution factor. Thus, 40 µL was selected for the subsequent experiments.

METHOD VALIDATION

Under the optimized experimental conditions, VALLME-BE method showed good linearity in the calibration range of 30 - 1000 µgL⁻¹, with coefficient of determination (R^2) of 0.9985, 0.9966, and 0.9987 for repaglinide, glibenclamide, and glimepiride, respectively. The limit of detection (LOD) for the extraction of repaglinide, glibenclamide, and glimepiride were found to be in the range of 0.41-1.66 µgL⁻¹ and limit of quantification (LOQ) were 1.38-5.54 µgL⁻¹, respectively. Table 1 summarizes the analytical performance of the established method.

TABLE 1. Method validation for the proposed VALLME-BE method

	Linearity range ($\mu g L^{-1}$)	r^2	LOD ($\mu g L^{-1}$)	LOQ ($\mu g L^{-1}$)
Repaglinide	30-1000	0.9985	0.41	1.38
Glibenclamide	30-1000	0.9966	1.04	3.45
Glimepiride	30-1000	0.9987	1.66	5.54

ANALYTICAL APPLICATION ON REAL SAMPLES

In order to evaluate the accuracy and applicability, the proposed extraction method was applied in environmental samples for the determination of repaglinide, glibenclamide, and glimepiride. All unspiked water samples showed no trace of repaglinide, glibenclamide, and glimepiride. The chromatograms of selected river and seawater were depicted in Figure 3.
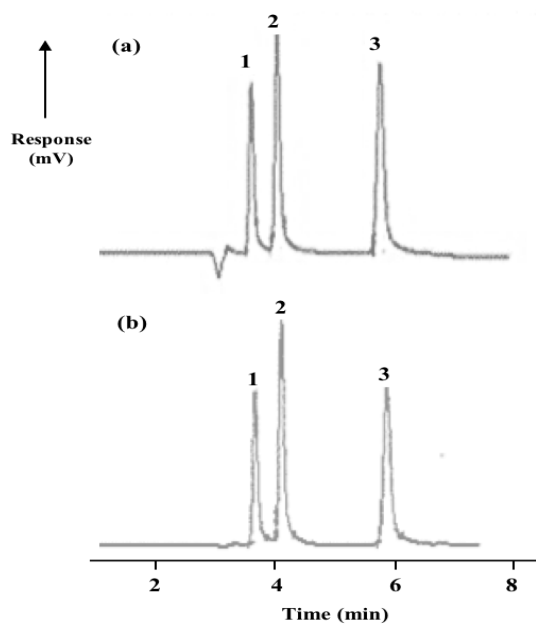

FIGURE 3. HPLC chromatograms of antidiabetic drugs subjected to VALLME-BE (a) spiked river (30 mgL^{-1}) and (b) spiked sea water (30 mgL^{-1}) under the optimized extraction conditions, (1) repaglinide, (2) glibenclamide and (3) glimepiride. Column; Thermo-Fisher Hypersil Gold ODS C18 (250 × 4.6 mm × 5μm), mobile phase; methanol and phosphate buffer (15 mM, pH 3.7), λ; 234 nm, flow rate; 1 mLmin^{-1}

The analytical results along with the recoveries for the spiked samples with known concentration (30, 500, 1000 µgL^{-1}) are listed in Table 2. The recoveries were acceptable (83.4 - 109.0%). The precision of the analytical procedure was determined using six replicates samples. The repeatability obtained was good (below 15%). It was found that the precision (% RSD) for three concentration levels (30, 500, and 1000 µgL^{-1}) was less than 9.5%.

TABLE 2. Recoveries and precision obtained by spiking river and seawater

C added (µgL^{-1})		Recovery (% X ± SD)			Precision (%RSD)					
					Inter-day			Intra-day		
		REPA	GLIB	GLIM	REPA	GLIB	GLIM	REPA	GLIB	GLIM
River[a]	30	89.2 ± 6.1	97.4 ± 7.1	98.9 ± 2.1	4.8	6.7	7.5	4.3	5.1	6.1
	500	101.6 ± 2.1	109.0 ± 6.1	87.5 ± 5.1	5.4	2.4	3.9	3.1	4.8	5.5
	1000	94.1 ± 3.1	100.0 ± 9.1	83.4 ± 5.1	6.2	7.2	4.5	7.7	3.5	8.9
Seawater[b]	30	98.1 ± 9.1	89.9 ± 7.1	90.6 ± 1.2	6.6	2.9	7.5	3.3	8.1	5.1
	500	105 ± 1.9	100.1 ± 6.7	87.9 ± 3.0	4.5	6.4	4.9	3.1	6.8	4.5
	1000	109 ± 2.7	109.0 ± 1.6	91.2 ± 2.6	2.3	3.2	2.5	3.7	1.5	4.9
Seawater[c]	30	103.9 ± 5.9	107.9 ± 6.9	90.0 ± 3.8	6.8	8.7	4.5	7.3	5.1	4.6
	500	96.9 ± 6.1	100.9 ± 1.5	104.6 ± 7.1	5.4	6.4	9.9	6.1	5.8	6.5
	1000	99.1 ± 8.1	87.3 ± 9.1	107 ± 4.5	3.2	5.2	7.5	4.7	8.5	4.9
Seawater[d]	30	88.7 ± 7.5	97.4 ± 4.1	106.9 ± 6.6	7.8	4.7	8.5	6.9	5.9	8.1
	500	88.3 ± 5.1	100.3 ± 3.1	90.1 ± 3.9	5.7	2.4	5.9	7.1	4.8	5.5
	1000	102.9 ± 7.5	99.8 ± 9.1	109.0 ± 1.1	3.2	6.2	7.5	4.7	8.5	4.9
Seawater[e]	30	99.0 ± 5.1	87.9 ± 5.2	104.6 ± 4.1	7.8	3.7	7.5	5.3	3.9	8.1
	500	103.9 ± 9.1	92.2 ± 3.1	98.7 ± 6.9	8.8	3.4	8.9	3.1	7.8	4.5
	1000	104.7 ± 2.1	84.0 ± 6.9	100.1 ± 2.1	4.2	3.2	3.8	4.7	3.5	5.1
Seawater[f]	30	99.9 ± 6.1	93.0 ± 1.3	100.7 ± 3.0	4.8	5.3	6.9	6.7	9.5	6.9
	500	83.3 ± 7.7	87.2 ± 3.3	109.0 ± 2.7	3.4	9.2	3.1	4.4	7.9	4.6
	1000	109.0 ± 6.9	95.0 ± 10.9	98.4 ± 6.1	9.2	7.2	9.5	5.9	9.4	6.2

REPA; repaglinide, GLIB; glibenclamide, GLIM; glimepiride
River water: a: Sungai Dua, Penang. Seawater: b: Bayan Lepas, Penang, c: Batu Ferringhi, Penang, d: Batu Maung, Penang, e: Padang Kota, Penang, f: Tanjung Bungah, Penang

COMPARISON TO PREVIOUSLY REPORTED METHODS

The performance of the developed VALLME-BE has been reviewed by a comparison with other reported preconcentration techniques for the extraction of repaglinide, glibenclamide, and glimepiride in literature, as summarised in Table 3. It was evident that the proposed method in the current work produced acceptable recoveries and in the range with other methods. The LOD and LOQ of the developed VALLME-BE was higher than the reported methods due to the different in instrumentation. However, most reported method require evaporation which is time-consuming unlike the proposed method. Moreover, the developed VALLME-BE method utilised low consumption of organic solvents as compared to other methods. The proposed method was a simple, low solvent consumption and rapid for the determination

of repaglinide, glibenclamide and glimepiride in water samples.

CONCLUSION

An interesting new analytical method has been developed for the HPLC-UV determination of antidiabetic drugs based on VALLME-BE. The proposed procedure combined sample clean and preconcentration into two steps (VALLME and BE) for the first time reported for the determination of repaglinide, glibenclamide, and glimepiride and the final extract can be directly injected for to the analysis into the HPLC column. The developed VALLME-BE method displays advantages such as simple, rapid, low cost, quickness, satisfactory sensitivity and environmental friendliness hence, it can be employed for a significant potential for extraction and the determination of these drugs.

TABLE 3. Comparison between the VALLME-BE and some reported method for the determination of REP, GLIB and GLIM in water sample

Analytes	Sample type	Extraction technique	Instrumentation	LOD (μgL^{-1})	LOQ (μgL^{-1})	Recovery (%)	Ref.
GLIB and 80 pharmaceutical compounds	Surface water	SPE	UPLC-QqLIT-MS	0.0005-0.0037	0.0017-0.0125	-	(Gros et al. 2012)
REP GLIB and 154 polar organic chemical contaminants	Wastewater	SPE	LC-MS-MS	-	0.0005 0.0100	-	(Loos et al. 2013)
MET GLIB VIL SIT PIO	Waste water River water Tap water	SPE	HPLC-QToF-MS	-	0.0082-0.032 0.0039-0.043 0.0004-0.0006 0.0031-0.0032 0.0011-0.0012	28-54 73-81 60-91 79-84 82-84	(Martín et al. 2012)
REP	Milli Q-water Surface water Waste water	SPE	LC-MS-MS	-	0.56 0.52 0.48	89 113-117 96-112	(Grabic et al. 2012)
GLIB	Effluent and surface water samples	SPE	UPLC-Q Exactive Orbitrap-HRMS	0.30	0.99	88.3	(Abdallah et al. 2019)
REP GLIB GLIM	Seawater and River water	VALLME-BE	HPLC-UV	0.41 1.04 1.66	1.38 3.45 5.54	83.3-109.0 84-109.0 83.4-109.0	This work

SPE: Solid phase extraction, UPLC: Ultra performance liquid chromatography, QqLIT: Quadrupole linear ion trap, MS: Mass spectrometry, REP: repaglinide, GLIB: glibenclamide, GLIM: glimepiride, VIL: Vildagliptin, SIT: Sitagliptin, PIO: Pioglitazone, HPLC: High performance liquid chromatography, QToF: Quadrupole time of flight, UV: Ultra-violet

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