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HOLLOW FIBRE SUPPORTED LIQUID MEMBRANE EXTRACTION OF DIPHENYLHYDRAMINE, CHLORPHENIRAMINE AND KETOPROFEN IN PHARMACEUTICAL PRODUCTS

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ABSTRACT. A simple sample pre-treatment method utilizing hollow fibre supported liquid membrane (HFSLM) was carried out on pharmaceuticals samples comprising of cough syrups (CS1 and CS2) and an anti-inflammatory product (AI). The active ingredients targeted in the extraction process were diphenylhydramine (DPH), chlorpheniramine (CPA) [(*S*)-γ-(4-chlorophenyl)-*N*,*N*-dimethyl-2-pyridinepropanamine maleate salt] and ketoprofen (KP) [(RS)2-(3-benzoylphenyl)-propionic acid] which were detected by a high performance liquid chromatograph (HPLC-UV/DAD) after HFSLM process. Factors controlling the efficiency of HFSLM extraction such as sample pH, stripping phase pH, enrichment time, stirring speed as well as the organic solvent used for the entrapment of these analytes were optimized to achieve the best selectivity and high enrichment factors. KP extracted from AI product had optimal pH of 10 for the stripping solution and 4 for sample with enrichment factors of 339. DPH had optimal pH of 12 (sample) and 3 for the stripping solution and had enrichment factors of 519. The optimal sample pH for CPA was 0.5 while the stripping phase pH was 13. The HFSLM technique showed 81% recovery of DPH from CS1, while for CPA from CS2 was 78 % and that of KP from AI was 80 %. The LODs obtained were 0.06 μg/L for all the compounds.

KEY WORDS: Hollow fibre supported liquid membrane, Enrichment factors, Pharmaceuticals, High performance liquid chromatography

INTRODUCTION

The quality of pharmaceutical products for human use has lately been given a special attention by the international community [1, 2]. A number of reports have pointed out the potential dangers of some ingredients in pharmaceutical products [3, 4]. Normally, the active ingredients in pharmaceutical products are rapidly absorbed, metabolized and excreted by the body once ingested due to the low half lives that they possess [5]. The active ingredients therefore provide a short window of effectiveness to prevent the drug levels from decreasing to an ineffective concentration [5].

These active ingredients play some important roles in the pharmaceutical formulations. For example, DPH is incorporated in a variety of pharmaceutical products, where it acts as an antihistamine [6, 7]. Histamine ($C_5H_9N_3$) is released by the body during several types of allergic reactions to take part in the body's immune responses [8, 9]. When histamine binds to its receptors on cells, it causes changes within the cells that lead to responses such as sneezing, itching and increased mucus production [10]. DPH as an antihistamine compete with histamine for cell receptors where they bind to the receptors and do not stimulate the cells. DPH is therefore used for the relief of allergic conditions and also has anticholinergic effects which treat motion sickness and Parkinsonism [11]. However, DPH is said to have the potential of causing sedation, dizziness, hallucinations and stomach distress and may also cause low blood pressure, palpitations and blurred vision [11]. Some severe side effects attributed to DPH include heart attack, coma and death [12-14]. It is also known to cause hypertension in sensitive individuals [15].

Another active ingredient found in some of the pharmaceutical products is ketoprofen (KP) which belongs to a group of drugs called nonsteroidal anti-inflammatory drugs [16]. It is used to

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reduce pain, swelling, and joint stiffness and works by reducing hormones that cause inflammation and pain in the body [17]. KP is known to have the potential of increasing the risk of life-threatening heart or circulation problems, including heart attack or stroke [16]. There is also concern over its phototoxicity and photosensitization potential as side effects [16].

The many health concerns of the active ingredients in pharmaceutical products have triggered scientists to devise effective monitoring mechanisms to ensure their safety. Numerous reports have suggested a number of methods and techniques to analyze specific ingredients in pharmaceutical products which are considered potentially a health hazard beyond certain limits [18]. Among these methods, solid phase extraction (SPE) [19] where-by Weigel *et al.* evaluated seven polymeric sorbents with regard to their ability to extract acidic, neutral, basic pharmaceuticals and estrogens simultaneously from water at neutral pH. Some of these sorbents performed very well (with recoveries between 70 and 110%) and some performed very poorly (with recoveries less than 30%). In another report Babić *et al.* [20] employed SPE in the extraction of pharmaceuticals in wastewaters and they reported recoveries ranging from 68.3 to 97.9% with RSD below 8.4%. Lin *et al.* [21] also developed an SPE method for the extraction of a range of pharmaceutical residues in waters. Recovery was reported to be ranging from 50 to 108% while RSD ranged from 1 to 10%. With these SPE methods we can see that though there are a number of compounds which extracts efficiently, still many other compounds extract poorly and may need another efficient extraction method.

Solid phase microextration (SPME) has also been reported in the extraction of pharmaceutical residues [22, 23]. Zambonin and Aresta used SPME in which a silica fibre (Supelco) coated with a 60 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB) was used [22]. Another method for extraction of pharmaceutical residues using SPME was developed by Yeung *et al.* [23]. In this approach three different SPME fibres (with different coating materials) were used. The coating materials were polydimethylsiloxane (PDMS), polydimethylsiloxane/ divinylbenzene (PDMS/DVB) and polyacrylate. Quantitation using a standard addition technique resulted in a recovery of 78.4 and 93.0% for the two compounds that were determined. So these methods too (SPME) may work best to some compounds and poorly to others, suggesting the need for more other robust extraction methods.

Other extraction methods such as liquid-liquid extraction (LLE) [24-26] have been reported as suitable for the extractions of basic, neutral or acidic molecules in different pharmaceutical samples. However these methods are known to have shortcomings in terms of economics and environmental concerns. As regards to the separation and detection, a number of approaches have been reported including capillary electrophoresis [27, 28]. Methods involving chromatography [18, 29] and mass spectrometry [30, 31] have widely been reported.

A simple, 'greener', efficient and cost effective method of sample preparation utilizing hollow fibre technique is being proposed as an alternative technique which will render sample preparation less demanding. We report here the application of HFSLM and HPLC-UV/DAD in the extraction and determination of active ingredients in pharmaceutical products. The structures of the compounds studied, their pK_a values and CAS numbers are given in Table 1.

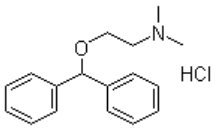
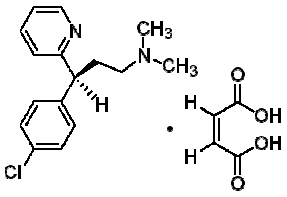
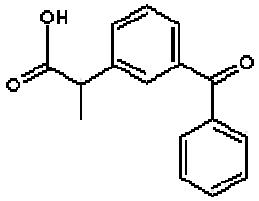
EXPERIMENTAL

Chemicals, reagents and materials

The cough syrups (CS1 and CS2) and an anti-inflammatory tube (AI) which contained DPH, CPA and KP, respectively, were purchased from local chemists and pharmacies around Durban City in South Africa. The organic solvent, isooctane, was purchased from Merck, (Switzerland). Standards of DPH, CPA and KP as well as the chemical extractant, tri-n-octylphosphine oxide

(TOPO) were purchased from Sigma-Aldrich (USA). A 100 µL Hamilton syringe was purchased from Perkin Elmer Instruments (USA). Hydrochloric acid (32 %) was purchased from N.T. Laboratory Supplies (South Africa) and sodium hydroxide pellets (98%) was from Saarchem (Krugerdorp, South Africa). HPLC grade solvents such as methanol were obtained from Merck (Germany). Mobile phases were filtered through 0.45 µM Millipore filters (Waters, USA). Hollow fibre was Q3/2 Accurel polypropylene hollow fibre membrane (200 µm wall thickness, 600 µm inner diameter, 0.2 µm pore size) purchased from Membrane GmbH (Germany). Working standard solutions (1 µg compound/mL) were prepared by appropriate dilutions of the stock solutions (1000 mg/L) and stored under refrigeration (4 °C).

Table 1. Structures of the compounds studied, their pK_a values and CAS numbers.

Compound	Structure	pK_a	CAS #
DPH		8.76	147-24-0
CPA		9.2	2438-32-6
KP		5.94	22071-15-4

Apparatus and chromatographic conditions

Shimadzu LC-20AT prominence HPLC with DGU-20A5 prominence degasser, SIL-20A prominence autosampler and SPD-M20A prominence DAD detector (Shimadzu Scientific Instruments, USA) was used for all analysis and determination of extracted compounds. The column used was an XTerra MS C_{18}, 3.0 mm x 150 mm x 3.5 µm (Waters, USA). The mobile phase comprised 75% methanol and 25% water. The isocratic mode of elution was used throughout at a flow rate of 0.3 mL/min with the injection volume of 5 µL.

HFSLM extraction procedure

The stripping buffer solutions were made following standard methods and the samples' pH was adjusted with 0.1 M HCl and 0.1 M NaOH. The hollow fibre was cut into 4 cm pieces and cleaned with acetone for about half an hour and sealed at one end by heating the end with a

hotplate. The open end was used to fill the lumen of the hollow fibre with the stripping buffer solution. The pores of the hollow fibre were then impregnated with isooctane containing 5% TOPO for a few seconds and thereafter rolled in water to remove any excess solvent. The impregnated hollow fibre filled with the stripping solution was thereafter immersed into 10 mL diluted pharmaceutical samples (1:5). The reaction was stirred at a constant rate (310 rpm) for 30 min. The HFSLM set up used is similar to the one reported previously [32].

RESULTS AND DISCUSSION

Choice of the organic liquid membrane

The influence of the organic solvent in the efficiency of the membrane extraction and the selectivity of enrichment of the analytes is vital in the HFSLM extraction process [33, 34]. For the efficient extraction and enrichment, the organic solvent must be stable, with low volatility and solubility in water and must have a large partition coefficient as compared to other interfering molecules. Large enrichment factors may be expected if a solvent has a high partition coefficient and stability in the porous membrane. In the extraction of active ingredients in pharmaceutical products, several organic liquids were tested and these include, isooctane, 5% TOPO in isooctane and toluene. The results of this optimization are shown in Figure 1.

Figure 1 shows that 5% tri-octylphosphine oxide (TOPO) in isooctane gave the highest enrichment factors. TOPO acts as a carrier/mediator in the extraction process where the electron lone pairs on the oxygen chelate to the molecules of the analyte of interest and therefore increases the efficiency of extraction [35]. Isooctane alone showed relatively high enrichment factors but lower than isooctane-TOPO mixture while toluene was the least. The reasons for low enrichment factors for toluene is probably due to instability of toluene in the pores of the hollow fibre. Toluene has a small solubility in water of 0.05 mg/L [36] and a large partition coefficient of 2.54 relative to isooctane which is 3.93 [37].

Optimization of sample and stripping solutions pH

The samples' pHs were optimized by keeping the stripping phase pH and all other factors constant while varying the sample pH for each compound. The sample pH range investigated for DPH was from 8 to 11 while that for CPA was from 8 to 14 and for KP was from pH 2 to 4. Each extraction was carried out for 45 min at constant stirring speed (310 rpm) with isooctane + 5 % TOPO as the organic phase.

The result for the sample and stripping solution pH optimization during the extraction of KP is shown in Figure 2. KP is a weakly acidic compound and for this reason the compound had its sample pH optimal at the acidic range where it displays the conditions for neutrality, which is a prerequisite for compounds to cross the hydrophobic organic membrane optimally [38].

In the pH optimization of stripping buffer solution the pH range investigated for KP was from pH 7 to 10 and the results are shown in Figure 2 which indicates that the optimum stripping solution pH for KP was 10. Since the KP is a weakly acidic compound it is expected to ionize at alkaline pH. The pK_a value of this weakly acidic compound KP is 5.94 which make the theoretical predictions for its stripping phase optimal pH to be around 9, however Figure 2 indicates that the optimum stripping solution pH for KP was 10. Since the KP is a weakly acidic compound it is expected to ionize at alkaline pH. The pK_a value of this weakly acidic compound KP is 5.94 which make the theoretical predictions for its stripping phase optimal pH to be around 9. However, the experimental pH value for the KP stripping phase obtained in this work is 10, which is in close agreement with the theoretical predictions.

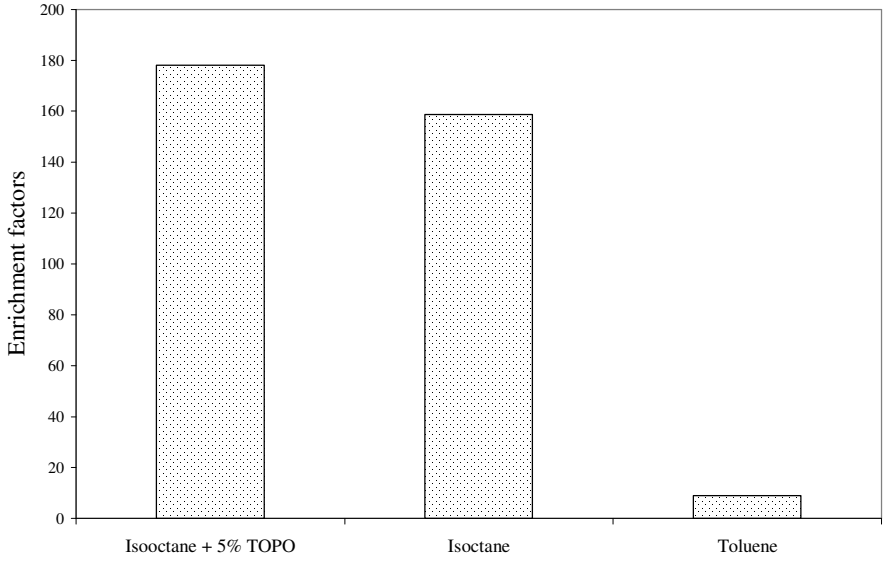


Figure 1. Choice of organic solvent in the HFSLM extraction of DPH.

DPH and CPA on the other hand are weakly basic compounds and therefore high sample pH keeps the compounds in their neutral form and hence extractable by the liquid membrane. The results in Figure 3 show that the optimal sample pH for DPH was 12 while that of CPA was 13 (data not shown).

In the pH optimization of stripping buffer solution the pH range investigated for DPH was from 3 to 6; and between 0.5-4 for CPA. Figure 3 shows the sample and stripping solution pH optimization results DPH.

The pK_a value for DPH was 8.74 and therefore, the theoretical optimal pH for DPH stripping solution was expected to be around 5. However, the experimental value obtained in this work for DPH was 3. The discrepancy could be accounted from the chemistry of the DPH dichloride used in this work (diphenylhydramine chloride) and this might have shaped the ionization behavior of DPH differently. The optimal pH for the CPA stripping solution from the experiment was found to be 0.5.

Optimization of time of extraction

The time of extraction was varied as it affects the mass transfer and hence the extent of enrichment. The results for the effect of enrichment time in the HFSLM extraction of DPH, KP and CPA are shown in Figure 4.

From Figure 4 it is clear that the enrichment factor increases with increase in time of extraction. Longer times of extraction allowed for longer time for contact between sample analytes and the liquid membrane and hence allowing more analytes to be trapped in the acceptor phase and thus more enrichment. This was evidenced by the increase in enrichment factors with time. After 45 min the enrichment factors slowed down a phenomenon that might as well be attributed to the fact that with long extraction times large amounts of analyte ions are produced in the acceptor phase which changes the pH of the acceptor phase. The pH changes to

less optimal conditions which decrease the efficiency of the extraction and hence the enrichment factor decreases.

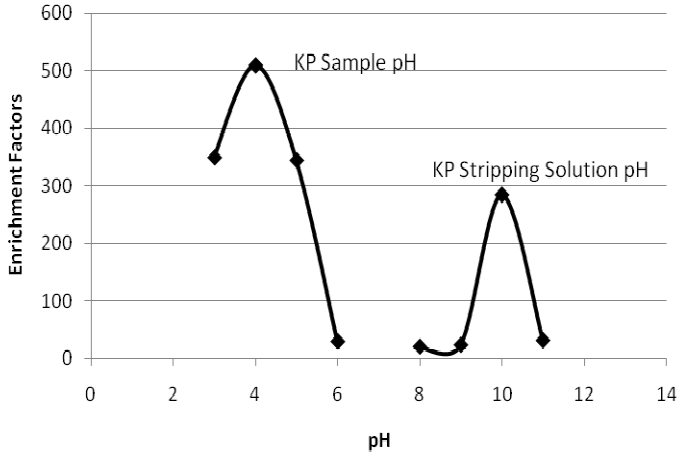


Figure 2. Optimization of sample and stripping solution pHs in the HFSLM extraction of KP from AI pharmaceutical sample.

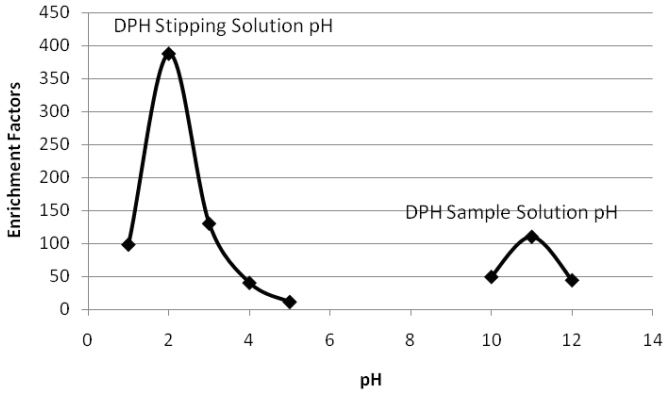


Figure 3. Sample and stripping solution pH optimization in the HFSLM extraction of DPH.

Optimization of stirring speed of the extraction

The effect of stirring speed on the enrichment factors in the HFSLM extraction of pharmaceutical samples is shown in Figure 5. An increase in stirring speed increases the rate of mass transfer, however, much higher stirring speeds are also known to negatively impact on the extraction process due to the fact that, higher speeds reduces the contact time between analyte species and the membrane.

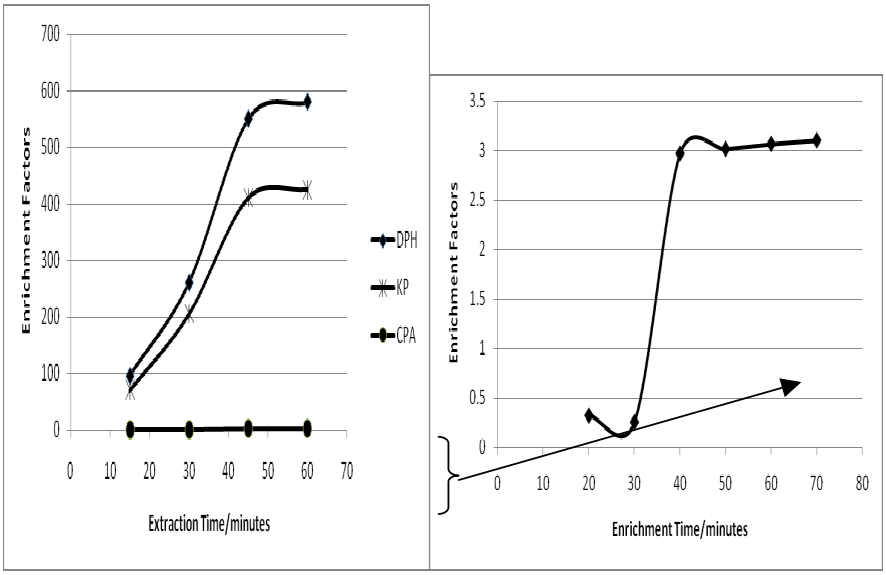


Figure 4. Optimization of extraction time for DPH, KP and CPA.

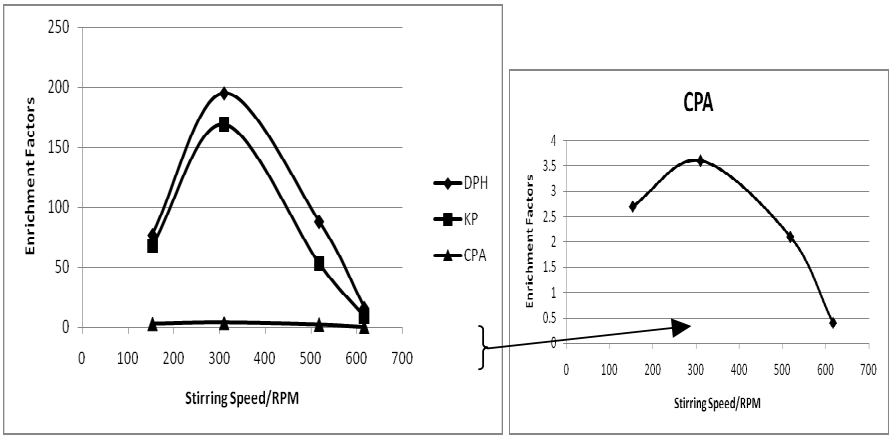


Figure 5. Optimization of stirring speed for DPH, KP and CPA.

High performance liquid chromatographic analysis of the extracts

All the compounds analyzed contain strong chromophores and therefore can absorb at the ultraviolet region of the electromagnetic spectrum. This made it possible to use HPLC with a UV-DAD detector to monitor the compounds after passing through a C18 column. The mobile phases used were methanol and water since the active ingredients are polar. This encouraged the active ingredients to elute faster and shorten the time of analysis. All the active ingredients eluted within first five minutes. Figure 6 shows the chromatographic elution profile for KP with a retention time of about 2.6 min.

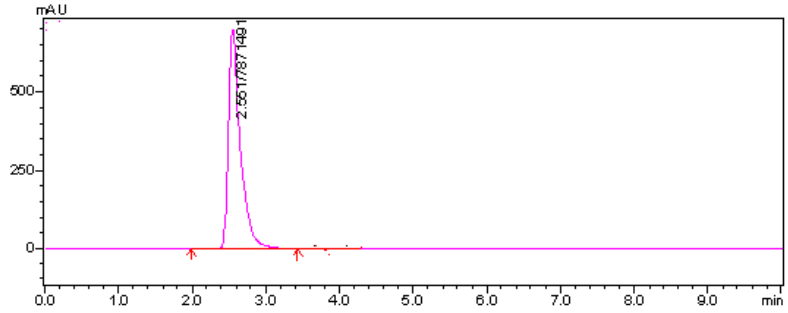


Figure 6. Chromatogram of KP after HFSLM extraction from AI anti-inflammatory sample.

Quantitation

The calibration graphs were used to calculate the concentrations in the stripping solutions which were then used to calculate the enrichment factors and the extraction efficiency.

Since the amount of DPH in CS1 as stated in the label was 12.5 mg/5 mL and the extracted amount was 10.15 mg 5 mL^{-1}, the percentage recovered was 81 %. KP was stated in the label as 0.35 g and the extracted amount was 0.28 g indicating an 80 % recovery. The recovery of CPA was found to be 78 %.

From the results above it can be deduced that the HFSLM extraction technique shows high selectivity to the active ingredients found in the pharmaceutical products analyzed as the percentage of active ingredients recovered were high.

Repeatability and reproducibility

To assess the reliability of the developed HFSLM method, reproducibility and repeatability in the extraction and enrichment processes were investigated. Reproducibility test refers to the absolute difference in the results between two test measurements performed in the same sample at two randomly selected times. Mathematically, reproducibility is defined by the following equation:

$$\text{Reproducibility} = 2.77(\sigma^2 t + \sigma^2/n)^{1/2} \quad (1)$$

Parameters $\sigma^2 t$ refers to the variance between samples over time, while σ^2 is the variance within the same sample, and n is the number of repeated measurements made on the same sample. On the other hand, repeatability was tested to investigate the absolute difference between two test results performed on the same sample, independent of time. Mathematically it is given by:

$$\text{Repeatability} = 2.77(\sigma^2)^{1/2}. \quad (2)$$

The set up for the repeatability and reproducibility was such that for each of the four spiked samples (DPH, CPA and KP) two sets of solutions (series 1 and series 2) were prepared. Each solution was then extracted twice (one after another successively) and this was done in similar manner over a period of four days. The results are shown in Table 2a and b. The data were analyzed statistically using SPSS v15.0 software.

The results of a comparative analysis in Tables 2a and 2b suggest the repeatability experiments gave close data whereas the reproducibility data is more dispersed. This means that with the developed technique, there is relative more reliability for the data performed on the same day than in different days. However this might have been contributed by the possible instability of the compounds under the experimental conditions.

Table 2a. Average enrichment factors and standard deviation obtained during the HFSLM of compounds.

Sample	Enrichment factors	Mean ± std error	Overall
DPH-1a	135	131 ± 5.6	127 ± 6.6
DPH-1b	127		
DPH-2a	125	122 ± 4.2	
DPH-2b	119		
KP-1a	116	113 ± 4.2	112 ± 3.9
KP-1b	110		
KP-2a	113	110 ± 4.2	
KP-2b	107		
CPA-1a	143	139 ± 5.6	137 ± 4.7
CPA-1b	135		
CPA-2a	138	135 ± 4.2	
CPA-2b	132		

Table 2b. Comparison for repeatability and reproducibility of compounds after their HFSLM extraction.

	1^{st} Day vs 3^{rd} Day			2^{nd} Day vs 4^{th} Day	
	Repeatability	Reproducibility		Repeatability	Reproducibility
DPH	15.5	26.0	CPA	15.5	20.8
			KP	11.6	16.6

Linearity of the HFSLM method and limits of detection (LODs)

The linearity of the method was investigated by spiking the samples with standards of the compounds at known concentrations and then extracting them using HFSLM. This procedure illustrating the linearity was carried out on all the samples by spiking in water at known concentrations (1, 10, 100 and a 1000 μg/L solution). Good linearity of the method for a wide range of concentrations was shown by the coefficient of linear regression (r^2) value which was of the order 0.99. This means that this method could be used for a wide range of concentrations of analytes with acceptable accuracy and precision.

The limits of detection (LODs) were estimated from the region where signal to noise ratio was typically equal to 3. The LODs obtained were 0.06 μg/L for all the compounds.

CONCLUSIONS

Hollow fibre supported liquid membrane extraction shows to be an effective extraction technique for the ingredients in pharmaceutical products due to the high enrichment factors and selectivity obtained. Factors affecting the efficiency of extraction were optimized by varying the sample pH as well as that of the stripping phase, extraction time, stirring speed and organic liquid solvent used as a membrane. Tri-n-octyl phosphine oxide (TOPO) showed to have a positive effect on the enrichment factors during the extraction of the active ingredients from pharmaceutical products.

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