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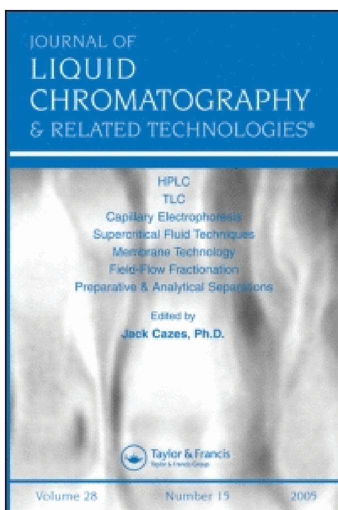

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SUPERCRITICAL FLUID EXTRACTION OF CLEVIDIPINE FROM A WATER BASED VEGETABLE OIL EMULSION

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ABSTRACT

A method was developed for the determination of clevidipine and some of its degradation products from a water based soya bean oil emulsion using supercritical fluid extraction (SFE). A trap packed with octadecyl silica (ODS) and stainless steel beads (ss) in the proportions 1:4 (v/v), with ODS closest to the restrictor, offered adequate trapping and recollection performance. The extractability of clevidipine (standard solution) was investigated using different sample support materials (hydromatrix, sea sand, filter paper, glass beads, and ss beads). Interactions were very small for ss beads but considerably stronger for, e.g., filter paper. On ss beads clevidipine could be extracted within a relatively short time (24 min) using mild conditions (91 bar, 0.50 g/mL, 4 mL/min, 40°C, 3% methanol, 25 thimble volumes swept). Filter paper required

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harsher extraction conditions (134 bar, 0.75 g/mL, 5% methanol, 40°C) to break interactions between clevidipine and the paper. However, filter paper gave a better distribution of the emulsion on the sample support than the more inert stainless steel beads. This resulted in higher recoveries (102% compared to 63% using steel beads) and better precision in the quantitative measurements of emulsion samples with a repeatability of 1.8% (RSD; n=8) for filter paper compared to 7.4% (RSD; n=8) for ss beads. Thus, filter paper was recommended for quantitative determination of clevidipine in emulsion samples. No degradation product of clevidipine could be found in the emulsion matrix. In order to detect the degradation products at least 3 mL emulsion has to be extracted to reach the limit of detection. At present such large volumes can not be sustained in the extraction thimble. The current maximum volume to be extracted is 1 mL.

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INTRODUCTION

Supercritical fluid extraction (SFE) is rapidly becoming a well-established method for sample preparation. Non-polar substances have been extracted from solid or semi-solid matrices, e.g., polyaromatic hydrocarbons (PAH) and polychlorinated biphenyls (PCB) from sediments,[1,2] and PCBs and dioxins from biological tissues.[3,4] The technique has also been applied to more polar substances in water solutions, e.g., phenols,[5] sulfonyl urea herbicides,[6] and phosphonates,[7] sometimes combined with solid phase extraction.[6] The results reported indicate a potential for the extraction of target compounds from matrices with high water content, such as emulsions. Typically, in the pharmaceutical industry, drugs insoluble in water are often dissolved in emulsions. Quality control of these products demands efficient and sensitive analytical methods for the drug itself, and related compounds. The sample work-up procedures, which are needed prior to the final analysis, are often time-consuming and requires a relatively large amount of organic solvents. Thus, SFE is an attractive alternative, since the technique is fast, easy to automate and significantly smaller volumes of hazardous solvents are used compared to normal organic solvent extraction.

Papers have been published regarding supercritical fluid extraction of drugs in pharmaceutical formulations, e.g., ibuprofen,[8] megestrol acetate[9] and felodipine[10,11] from tablet matrices. A somewhat different approach has been reported for the determination of acyclovir[12] and polymyxin B[13] in ointments by so called inverse SFE. With this technique the carbon dioxide soluble matrix is extracted, leaving the insoluble polar analyte of interest behind.

To analyze components present in emulsion matrices, the emulsions needs to be cracked in order to make the analyte available. This procedure can be performed with various methods, e.g., dilution in a suitable solvent,[14,15] freeze drying, heating, or treatment in ultrasonic bath.[15] With the unique penetration and diffusion properties of supercritical carbon dioxide, extractions with SFE technique are possible without any sample pretreatment. This has been shown by Hedrick and Taylor,[16] who extracted sulfa methazine spiked in whole milk with a recovery of 95%, and by Mulcahey and Taylor[17] in their extraction of sulfa methoxazole and trimetoprim from SeptraInfusion (an aqueous based matrix).

The goal of this work was to efficiently extract the drug clevidipine, from a water based soya oil emulsion containing large amounts of fat, which also puts high demands on the selectivity of the extraction method.

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EXPERIMENTAL

Equipment

The extraction system consisted of a Hewlett-Packard 7680T SFE unit (Wilmington, DE, USA) and a Hewlett-Packard 1090 LC pump for the introduction of modifier into the system. To control the SFE system a Hewlett-Packard 386/25N personal computer with a Windows based software (Hewlett-Packard, G1225C, version 4.01) was used. Hewlett-Packard standard 7 mL extraction thimbles were used in all experiments.

Two Hewlett-Packard standard traps were used, packed with stainless steel beads (ss) and ODS (octadecyl silica), respectively. The rinse fractions from the trap were collected in standard (1.8 mL) sample glass vials (Chromacol Ltd., Welwyn Garden City, UK). The nozzle temperature of the SFE unit was set to 5°C above the trap temperature.

Separation and quantitative analysis of the SFE extracts was performed on an LC system consisting of a Kontron MSI 660 auto sampler (Kontron Instruments SPA, Milano, Italy), equipped with a 20 µL injection loop, a Waters 501 LC pump (Waters Associates, Milford, MA, USA), and a LDC Spectromonitor III UV detector (LDC, Riviera Beach, Florida, USA) with the wavelength set to 240 nm. The column used for the LC analysis was a reversed phase ODS (Nova-Pak C18 60Å 4µm, 3.9 x 150 mm, Waters).

For the collection of chromatographic data a PC (Hewlett-Packard 486/50 VL) with Borwin (JMBS Developments, Le Fontanil, France) chromatographic data system software (version 1.21) was used. All calculations and graph plotting were done in Excel 5.0 for Windows (Microsoft Corporation, Redmond, WA, USA) or in KaleidaGraph version 3.06 for Windows (Synergy Software, Reading, PA, USA).

Chemicals

The extraction gas in all experiment was pure carbon dioxide (4.8 or 5.2 grade, >99.998% and >99.9992%, respectively; AGA Specialgas, Stockholm, Sweden).

Stainless steel beads (316 L, Ø=300-385 μm) were supplied by Anval (Torshälla, Sweden). The ODS material (Part No. 79903-85031) was obtained from Hewlett-Packard. Glass beads, o.d. ca 1mm was delivered by KEBO Lab (Spånga, Sweden). Hydromatrix was supplied from IST (Hengoed, Mid Glamorgan, UK).

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Methanol (HPLC) and acetonitrile (HPLC), were purchased from LAB-SCAN (Dublin, Ireland). Sodium dihydrogen phosphate monohydrate (p.a.), sodium hydroxide (p.a.), ortho-phosphoric acid 85% (p.a.), and sea sand (p.a.) were obtained from Merck (Darmstadt, Germany). Ethanol (95%) was delivered from Kemetyl (Stockholm, Sweden). All water used was p.a. quality or better.

Clevidipine and its degradation products H 152/81 and H 324/78, and felodipine (internal standard), were all prepared in house at Astra Hässle (Mölndal, Sweden). Emulsion samples containing water, vegetable oil, and 0.3 mg of clevidipine were supplied by Astra Hässle. The chemical structures of clevidipine and felodipine are shown in Figure 1.

Preparation of Stock Solutions

The LC mobile phase was prepared by mixing 4 volumes of acetonitrile, 2 volumes of methanol and 4 volumes of buffer. The buffer was prepared by mixing 15 mL of phosphoric acid (1 M) with 100 mL of sodium dihydrogen phosphate (1 M). This mixture was then diluted to 2000 mL and the pH value was checked to 3.0±0.1, and if necessary, adjusted with phosphoric acid or sodium hydroxide. Before use, the mobile phase was degassed in an ultrasonic bath for 10 minutes.

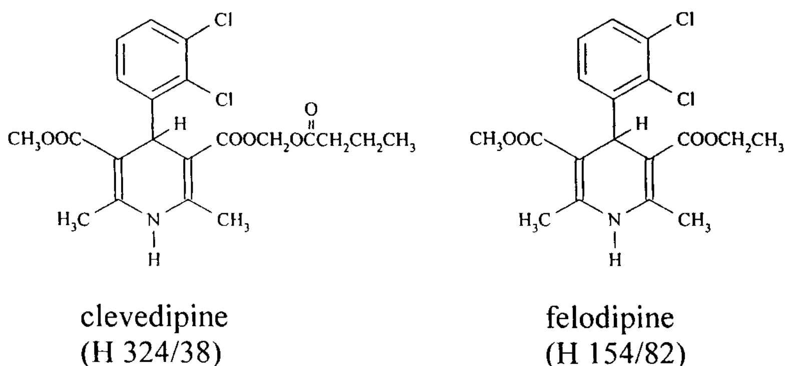

Figure 1. Structure of clevidipine and the internal standard felodipine.

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The rinsing solvent used in all experiments was prepared by mixing 4 volumes of acetonitrile, 2 volumes of methanol, and 4 volumes of water. This mixture (the LC mobile phase with the buffer part replaced with water) was chosen in order to obtain extracts with a suitable solvent strength for injection in the chosen LC system, while at the same time preventing crystallization in the switching valve of the SFE unit.

Stock standard solutions were made by dissolving 12.5 mg clevidipine, 5 mg H 152/81, and 5 mg H 324/78, respectively, each in 25 mL of ethanol. The internal standard solution was prepared by dissolving 25 mg felodipine in 50 mL of ethanol.

All solutions were placed in an ultrasonic bath for 15 minutes. The standard solutions were stored at 8°C in dark bottles to prevent degradation

Experimental Procedure

From the emulsion samples, stored at 8°C, an aliquot of 1 mL were taken with a single use syringe (Asik, Rødby, Denmark). 100 µL of this aliquot was subsequently pipetted on to stainless steel beads in the extraction vessel. To mix the stainless steel beads and the emulsion, the extraction thimble was shaken on a vortex mixer (Reax 2000, KEBO, Spånga, Sweden) for 1 minute.

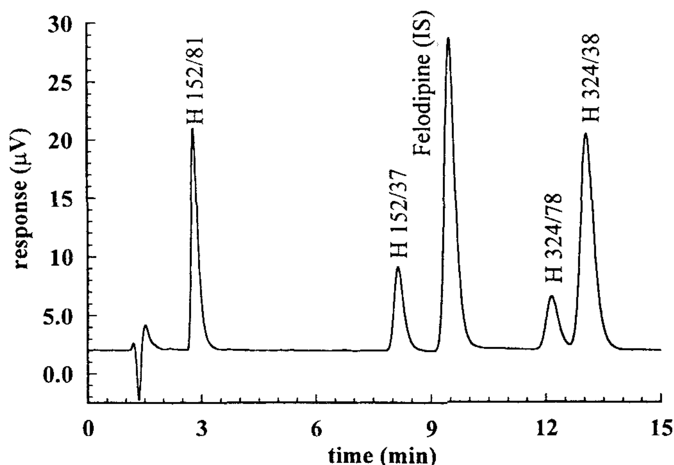

Figure 2. A typical chromatogram of the standard solution.

In all extraction experiments 50 μL of the internal standard solution was pipetted into the sample vials in the fraction collector of the SFE unit. Prior to LC analysis the vials were gently shaken. Pure substance samples was applied by pipetting 50 μL of the different standard solutions on to a folded filter paper (diameter 55 mm, Munktells, Grycksbo AB, Stora Kopparberg, Sweden) or on to 3.5 g stainless steel beads corresponding to a volume of 1 mL.

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RESULTS AND DISCUSSION

Chromatography

A typical standard chromatogram is shown in Figure 2. Good resolution is obtained between the substances of interest.

Optimization of the Trapping Procedure

The elution characteristics of two standard traps, packed with octadecyl silica (ODS) or stainless steel beads (ss), was first evaluated. For this purpose, the following operations were undertaken: (1) removing the trap, (2) application of 50 μL of the clevidipine stock standard solution at the nozzle

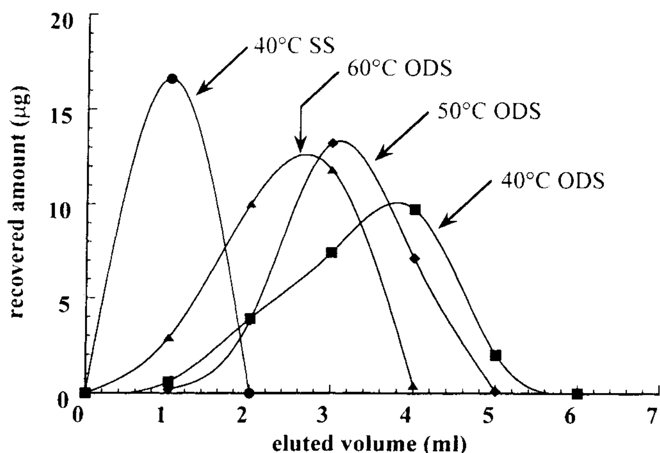

Figure 3. Amount recovered clevidipine versus elution volume for different traps and temperatures. Trap packing material; Octadecyl silica (C18), Stainless steel beads (ss).

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outlet, (3) repositioning the trap and (4) initiating the rinsing process. In the Hewlett-Packard unit, rinsing is preceded by a pressurizing/depressurizing sequence. During the depressurizing step the gas flows through the trap, which means that the solvent in the added clevidipine sample is removed.

Elution was then performed at different temperatures (40-60°C) using the mobile phase (without buffer) as the rinsing solvent. The relation between eluted amount of clevidipine and elution volume is shown in Figure 3.

For the stainless steel trap, a satisfactory elution volume (less than 2 mL) is already obtained at 40°C. At the same temperature an elution volume of 6 mL is needed for full recovery of the target analyte from the ODS trap. Rinsing at a temperature of 50°C reduces this volume to 5 mL and a further increase to 60°C reduces it to 4 mL.

To be able to combine the superior trapping efficiency of the ODS material and the advantageous elution characteristics of the ss-packing, a new trap was dry packed with 0.21 g of ODS and with the remaining volume filled with ss-beads (Figure 4).

The elution profile for the combined ODS/ss trap was investigated in the same manner as described above. Results are shown in Figure 5.

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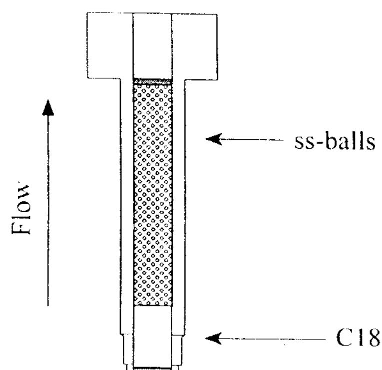

Figure 4. Schematic picture of combined trap containing octadecyl silica (ODS) and stainless steel beads (ss) in proportions 1:4 (v/v).

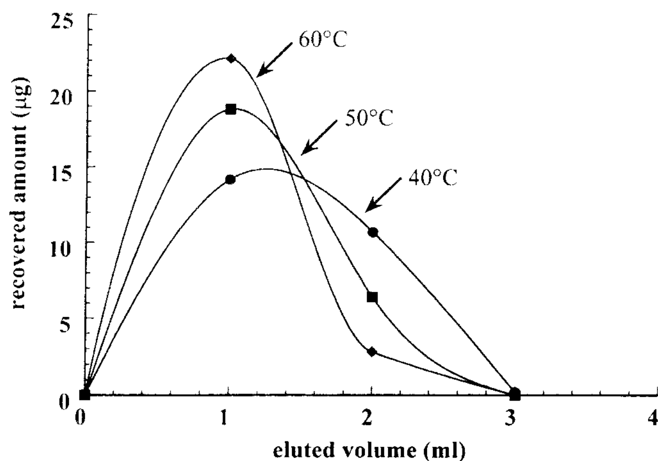

Figure 5. Clevidipine recovery versus elution volume for combined ODS/ss trap at three different temperatures (40, 50, and 60°C).

As expected, the elution profiles are sharper at higher temperatures for this two phase trap. To reduce the risk of solvent evaporation in the trap, 50°C was chosen as the rinse temperature in the following experiments.

Extractability of Clevidipine and its Degradation Products

In the optimization of the SFE procedure it is very important to monitor the extractability of the pure analyte in the supercritical fluid, prior to extraction of real samples.[18] The most common way to do this is to extract the substance of interest from some kind of inert material. Filter paper has proven to be suitable for this type of investigation.[19]

In order to keep the co-extraction of lipids from the emulsion at a minimum, the extraction temperature[20] and the density of the supercritical carbon dioxide should be kept as low as possible.[19] Hence, a temperature of 40°C was used throughout this work. The trap temperature and the flow rate was arbitrary set to 40°C and 4.0 mL/min, respectively. Four different pressures were tested, i.e., 77 (0.25 g/mL), 91 (0.50 g/mL), 134 (0.75 g/mL), and 281 bar (0.90 g/mL).

When extracting clevidipine from filter paper, the recovery was also very low (ca 35%) for the highest pressure investigated. Howard et al. extracted felodipine from cotton balls with pure carbon dioxide and achieved a full recovery with 320 bar and 45°C.[21] The low recovery in our experiments, when compared to the Howard paper, could either be due to poor solubility or due to analyte interactions with the matrix.

To test the latter assumption, an aliquot of the clevidipine standard solution was applied on to sea sand, glass beads, stainless steel beads, and Hydromatrix. Extraction was performed at 91 bar (0.50 g/mL). The results are shown in Figure 6.

It is clear that the two most inert materials, glass beads and ss-beads, give acceptable recoveries. The shape of the curves for those two materials indicate that the solubility of clevidipine in the supercritical fluid is sufficient. Hence, the low recovery obtained for the other materials most certainly can be attributed to strong analyte/matrix interactions.

Lowered recovery of analytes applied on filter paper, compared to extraction from stainless steel surfaces, has previously been observed by Karlsson et al.[3] The recovery of the drug budesonide was decreased from 80% when applied on stainless steel, compared to less than 30% using filter paper.

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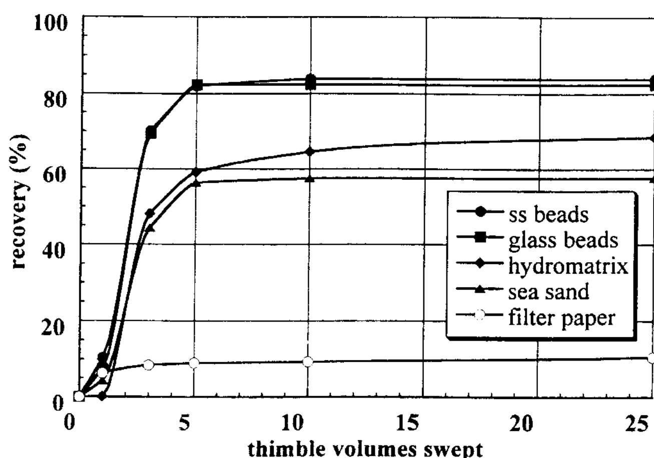

Figure 6. Recovery versus thimble volumes swept for various sample supports. Extraction conditions: 91 bar, 40°C, 4 mL/min, trap temperature: 40°C. All profiles are sequential extractions of a single sample (n=2).

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In the latter case, the addition of 5% methanol to the extraction fluid increased the recovery to over 80%. On the other hand, no such effects were observed, when Mågård et al.[19] applied androstenone on filter paper, suggesting that molecular structure is of great importance for the magnitude of the analyte/matrix interaction. One main difference between budesonide and androstenone is that hydroxyl groups are present in budesonide. These groups have the ability to form strong hydrogen bounds with the cellulose in the filter paper. Thus, choosing support materials is an important step in the method development procedure.

Since it is known that glass contains silanol groups that might interact with some of the functional groups in the other investigated compounds, stainless steel beads were used in all further extractability experiments. Extractability experiments of degradation products from clevidipine were performed using the same parameters as described above with 91 bar (0.50 g/mL) and stainless steel beads in the extraction vessel. Results are shown in Figure 7.

Almost full recovery (more than 85%) is accomplished for all substances except for the somewhat acidic and more polar H 152/81 (37%). To increase the extractability of H 152/81, methanol was used as a modifier.

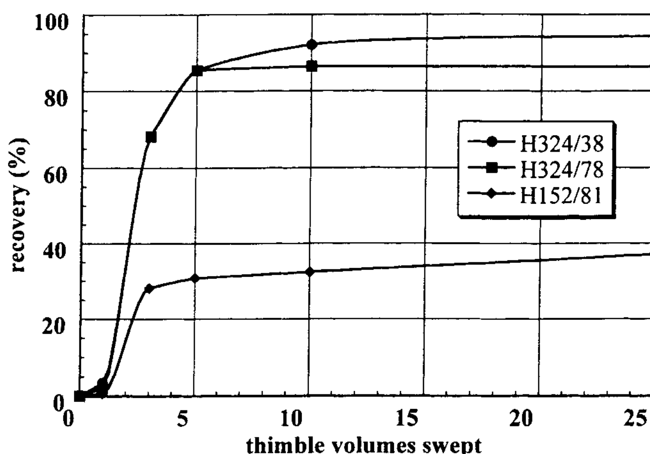

Figure 7. Plot of the recovery versus thimble volumes swept for clevidipine and two degradation products. Extraction conditions: 91 bar, 40°C, 4 mL/min, trap temperature: 40°C. All profiles are sequential extractions of a single sample (n=2).

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In order to prevent the methanol to condense in the collection step, the trap temperature was raised from 40°C to 70°C (approximately 5°C above the boiling point of the modifier). Figure 8 shows results using 3% of methanol as modifier in supercritical carbon dioxide.

Clearly, 3% methanol is sufficient to give a good recovery of H 152/81 without any significant change in the recovery of the other investigated substances. Further addition (5%) of methanol lowered the recovery for H 324/78 (not shown). A possible reason for this, is that a small amount of water is introduced together with the methanol. When water is exposed to carbon dioxide, protons are formed and hence the pH is lowered.[22] It was observed by Hedrick and Taylor[16] that a low pH (3.5) is formed when water is mixed with supercritical carbon dioxide. At this pH none of the three nitrogen containing bases could be extracted. Protonation makes the bases more dissolvable in the water phase and decreases their ability to be extracted by carbon dioxide.

Extraction of Clevidipine from Emulsion

The first goal was to quantitatively extract clevidipine from the emulsion matrix. Since the active substance occurs in a relatively large amount, only a small volume of emulsion is needed for the analysis. In this case, 100 μL is

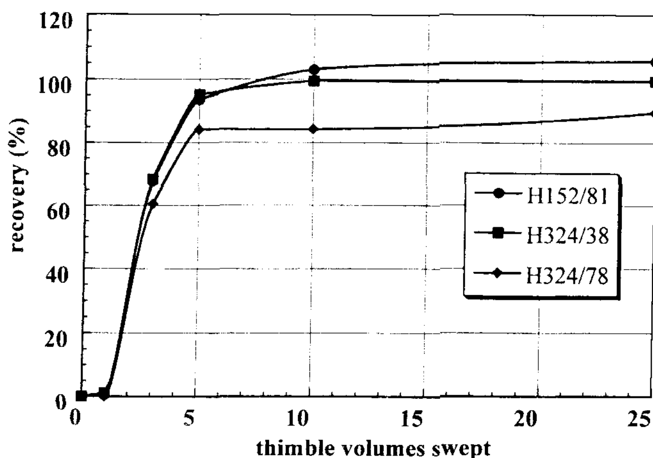

Figure 8. Recovery versus thimble volumes swept for clevidipine and two degradation products. Extraction conditions: 91 bar, 40°C, 4 mL/min, 3% methanol, trap temperature: 70°C. All profiles are sequential extractions of a single sample (n=2).

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sufficient for the quantitation of clevidipine. When applying the emulsion on to the stainless steel beads, one single drop of emulsion was formed due to lack of capability to wet the matrix surface. To obtain a better distribution of emulsion on the surface, the extraction thimble was shaken using a vortex shaker. Extraction of emulsions using conditions which gave complete recovery for standard solutions (91 bar, 0.50 g/mL, 3% methanol, trap temperature: 70°C, 25 thimble volumes swept) gave low recovery (63%) and relatively poor repeatability (RSD(%) 7.4, n=5). A higher trap temperature during extraction was also tested (80 and 90°C) in order to exclude a possible break-through caused by modifier condensation. However, this approach did not improve the recoveries further. A plausible explanation for the decrease in recovery is that micelles from the emulsion (with included analyte) will be transported from the extraction vessel and pass the trapping tube without being trapped.

To improve the repeatability of the extraction, filter paper was tried instead of stainless steel beads in the extraction vessel. Capillary forces in filter paper helps spreading the emulsion more evenly. This, however, requires stronger extraction conditions in order to overcome the analyte/matrix interactions as demonstrated in Figure 6. Recovery experiments at a pressure of 134 bar (0.75 g/mL) and a modifier concentration of 5% methanol is shown in Figure 9.

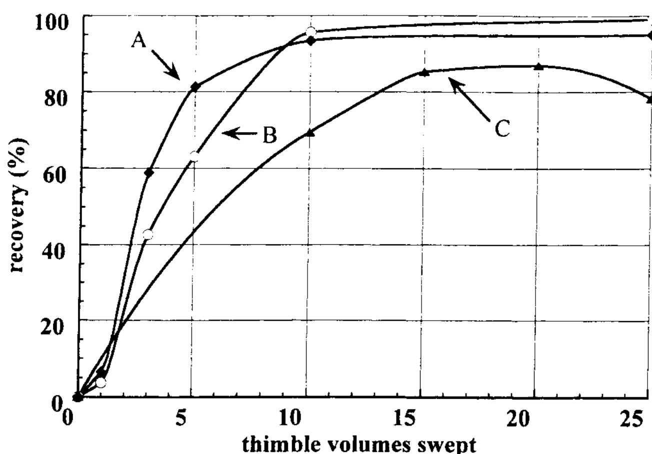

Figure 9. Recovery versus thimble volumes swept for clevidipine applied on filter paper. A: Standard solution. Sequential profile using a single sample (n=2). B: Emulsion. Sequential profile using a single sample (n=2). C: Emulsion. Continuous extraction using one sample for every single point (n=2). Extraction conditions: 134 bar (0.75 g/mL), 40°C, 4 mL/min, 5% methanol, trap temp: 70°C.

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In Figure 9A and 9B one sample is used to obtain the recovery profile. Each point on the curve represents a fractionated extraction followed by elution of the analyte collected on the trap during this extraction. In Figure 9C, the points represent different samples, which have been extracted using different amounts of extraction fluid. Figure 9A shows that quantitative recovery of clevidipine in standard solution pipetted on filter paper is obtained with a consumption of 10 thimble volumes, corresponding to an extraction time of 14 minutes.

For the emulsion sample in Figure 9B complete recovery could be achieved with 25 thimble volumes swept or 36 min. However, in Figure 9C, where emulsion samples were extracted continuously the recovery is lowered and even drops from ca 87% to 78% from 20 to 25 thimble volumes. In the continuos mode, all 25 thimble volumes are swept in one extraction step. The decrease in recovery is probably due to a transport of the analyte by condensed modifier in the trap.

The reason for modifier condensation at a trap temperature of 70°C (b.p. methanol 64.7°C) could be explained by insufficient heat transport properties in the trap packing material. To circumvent this, the trap temperature was

increased another 10°C to 80°C. With sequential extraction periods (1, 2, 2, 5, and 15 thimble volumes) the risk of a possible breakthrough is substantially lower since the trap is rinsed after each, relatively short, extraction step.

With the new trapping parameters, i.e., 134 bar (0.75 g/mL), 5% methanol, and trap temperature 80°C, 8 samples were analyzed to check the repeatability of the SFE/LC method. Mean recovery for the extracted samples was found to be 102% compared to the results from a traditional liquid-liquid extraction method set to 100%. The repeatability was excellent, with a relative standard deviation of 1.8%.

Thus, quantitative extraction of clevidipine is possible using filter paper as sample support and relatively high pressure and modifier concentration. This means that selectivity is sacrificed towards fat, which is co-extracted to a higher extent at the harsher extraction conditions. However, since only small sample volumes (100 μl) are needed, the small amounts of fat injected on the LC column do not cause any problems with final analysis. One drawback by using high amount of modifier (5%) is that the extraction efficiency for H 324/78 decreases.

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Extraction of Degradation Products from Emulsion Samples

The method used here, which is designed for the target substance, might also be applied to degradation products. However, in the case of clevidipine emulsion batch at our disposal, the concentration of the degradation products are too low; in this case requiring a sample volume of 3 mL to exceed the detection limit. The maximum sample volume which can be handled with the present approach is 500 μl. This means that the method needs to be modified if small amounts of degradation products are to be determined.

CONCLUSION

The developed method for the extraction of clevidipine gives quantitative recovery and good precision. The applied procedure can most probably be extended to the extraction of other similar drugs in emulsion formulations.

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