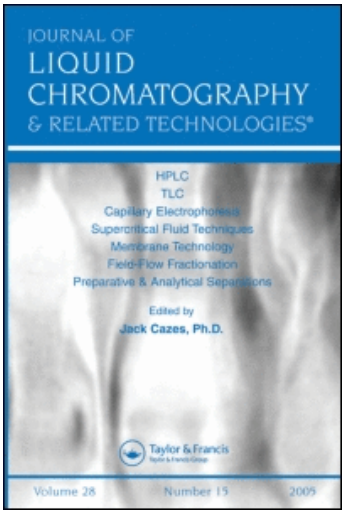

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DETERMINATION OF FELODIPINE IN TABLETS USING ACCELERATED SOLVENT EXTRACTION

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ABSTRACT

A method for the content determination of felodipine from tablets using accelerated solvent extraction (ASE) was developed. Acetonitrile, methanol and a mixture of the two (2:1, v/v) were evaluated as the extraction solvents, at two different temperatures, 50°C and 100°C, for ground tablets. Acetonitrile gave the fastest extraction and methanol the slowest at both temperatures. No difference in extraction efficiency was observed between the two temperatures. To minimize sample handling, extraction of whole tablets was performed using the same parameters as for the ground tablets. 100°C gave higher recoveries than 50°C.

However, quantitative recovery could not be achieved within an acceptable extraction time due to slow mass transfer in whole tablets. Hence, a different approach was used where tablets were crushed in a filter paper inside the extractor cell using a pair of flat pliers. This approach provided a straightforward way of transferring the entire tablet to the cell without losses. The final method was capable of extracting over 98% of felodipine within 15 minutes of static extraction at 50°C and 1500 psi, using acetonitrile as the solvent. No target analyte breakdown was observed at these conditions.

INTRODUCTION

The determination of the active compound content in tablets is a fairly common operation in pharmaceutical analysis. Typically, the tablets are ground using a mortar and pestle prior to the extraction step that precedes the final determination. The extraction step involves liquid extraction, and is sometimes speeded up by ultrasonication. Nevertheless, the operation is performed manually and is often time consuming. Hence, there is an obvious need for automated sample preparation methods.

Accelerated Solvent Extraction (ASE) is a novel technique employing organic solvents at elevated temperatures (50-200°C) and pressures (7-20 MPa) for the automated extraction of analytes. Thus far, applications have been limited to solid environmental samples.[1-4] Williams and co-workers, though, used ASE for the extraction of drugs from rodent food.[5] In a recent paper by Richter et al.[6] the effects on the extraction efficiency at elevated temperatures and pressures were investigated. As expected an increase of these two parameters increased the recovery for a given extraction time.

A typical ASE sequence consists of 6 steps: (1) Loading of the sample in extraction cells, (2) filling of the extraction cell with solvent, (3) heating and pressurization of the cell, (4) addition of clean solvent, (5) purging (using a suitable gas) of solvent from the cell in a pulsed manner, and (6) analysis of the extracts. ASE is claimed to be fast and easy to automate. As in supercritical fluid extraction (SFE) rapid extractions occur due to increased analyte mass transfer. Another similar feature to SFE is the technical solution admitting a large number of samples to be automatically, sequentially processed. A distinct advantage with ASE is that optimization procedures are usually not elaborate since the same solvents as in traditional extractions are used.

In this paper we have outlined the possibility of using ASE for the determination of felodipine in a solid tablet matrix, emphasizing temperature effects on recovery and analyte degradation. To the best of our knowledge, this is the first evaluation of the ASE technique in the analysis of a tablet formulation.

EXPERIMENTAL

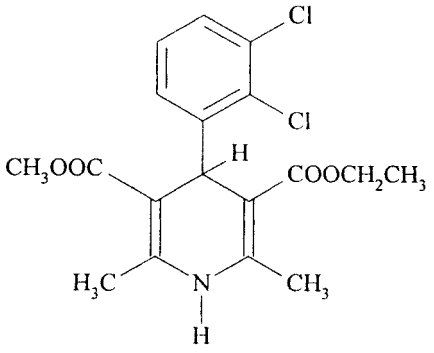
Equipment

Extractions were performed using an ASE 200 Accelerated Solvent Extraction system (Dionex Corporation, Sunnyvale, CA, USA). Analysis of the ASE extracts were performed on an LC system consisting of a Kontron MSI 660 auto sampler (Kontron Instruments SPA, Milan, Italy) equipped with a 20 µL injection loop and a Waters 501 LC pump (Waters Associates, Milford, MA, USA). The detector was an LDC Spectromonitor III (Division of Milton Roy Co, Riviera Beach, FL, USA) with the wavelength set to 240 nm. The column used for the analysis was a reversed phase ODS (Nova-Pak C18 60Å 4 µm, 3.9 X 150 mm, Waters). A PC (Hewlett-Packard 486/50 VL) with Borwin (JMBS Developments, Le Fontanil, France) chromatographic data system software (version 1.21) was used for the collection of chromatographic data. 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).

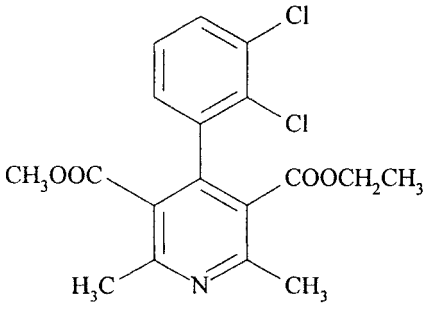
Chemicals

Nitrogen (of "Plus" quality) was delivered by AGA gas AB (Sundbyberg, Sweden). Acetonitrile and methanol, both of HPLC grade, were purchased from LAB-SCAN (Dublin, Ireland). The water was of p.a. quality or better. Sodium dihydrogen phosphate monohydrate, sodium hydroxide, and ortho-phosphoric acid 85%, all of p.a. quality, were obtained from Merck (Darmstadt, Germany).

Ethanol (95%) was delivered from Kemetyl AB (Stockholm, Sweden). The 10 mg felodipine tablets, the felodipine reference as well as its oxidative degradation product H 152/37, were from Astra Hässle (Mölndal, Sweden). The chemical structures of felodipine and H 152/37 are shown in Figure 1.



felodipine



H152/37

Figure 1. Chemical structure of felodipine and the degradation product H 152/37.

The LC mobile phase was prepared by mixing acetonitrile, methanol and buffer in the proportions 4:2:4 (v/v/v). Preparation of buffer was done by mixing 100 mL of sodium dihydrogen phosphate (1 M) with 15 mL of phosphoric acid (1 M), and diluting the mixture to 2000 mL with water. The pH value of the mixture was checked to 3.0 ± 0.1. Adjustments were made with phosphoric acid or sodium hydroxide if required. The mobile phase was placed in an ultrasonic bath and degassed for 10 minutes prior to use.

A stock standard solution of felodipine was prepared by dissolving 2.5 mg/mL in ethanol followed by ultrasonication for 15 minutes. This solution was used to prepare reference solutions in the concentration range of 1 to 100 μg/mL. These were stored in darkness at 8°C to prevent degradation.

Procedures

The Dionex ASE unit contains a number of extraction cells (which can be pressurized to 20 MPa and heated to 200°C) and an integrated collection unit with 26 collection vial positions. The system is automated and 24 samples can be processed sequentially. For our applications, samples were loaded in Dionex standard stainless steel cells with a volume of 11 mL. A filter paper (Ø=20 mm, Waters) was routinely put at the exit of the cell to prevent clogging of the metal frit due to tablet matrix being co-extracted. In some experiments filter paper from another vendor (Ø=55 mm, Munktells, Grycksbo AB, Stora Kopparberg, Sweden) and hydromatrix IST (Hengoed, Mid Glamorgan, UK) was also present in the extraction cell. The extraction starts with a filling step when the solvent of choice is pumped into the cell. The next step is preheating, which has a length of 5 minutes at 50°C. This step assures that the sample is completely heated in the extraction oven before the static extraction starts. After the static extraction, when the cell is held at a constant pressure and temperature, the pressure is released. The extract is then collected in 25 mL glass vials and the extraction cell is rinsed with fresh solvent. This rinsing volume is referred to as the flush volume and the default value, as set by the software, is 60% of the extraction cell volume. Finally, the extraction cell is purged with pure nitrogen for 1 minute to assure that the solvent is completely transferred to the collection vial.

LC analysis of the extracts was performed by taking an aliquot of 50 or 100 μL from the collection vial and diluting it to 1.8 mL. If necessary, the samples were filtered using a glass syringe and a MF-Millipore filter 1.2 μm (Millipore, Molsheim, France). Felodipine was quantified using seven standards (1, 2, 5, 10, 20, 50, and 100 μg/mL). A linear regression plot with the intercept close to the origin was obtained in the investigated concentration range (R=0.999).

For comparison, a method using ultrasonication and LC was used. In short, with this scheme, tablets are pulverized in a mortar and ultrasonicated for 5 minutes with acetonitrile. Methanol is added and another 30 minute ultrasonication session is performed. The final LC analysis of the extract is performed as described above.

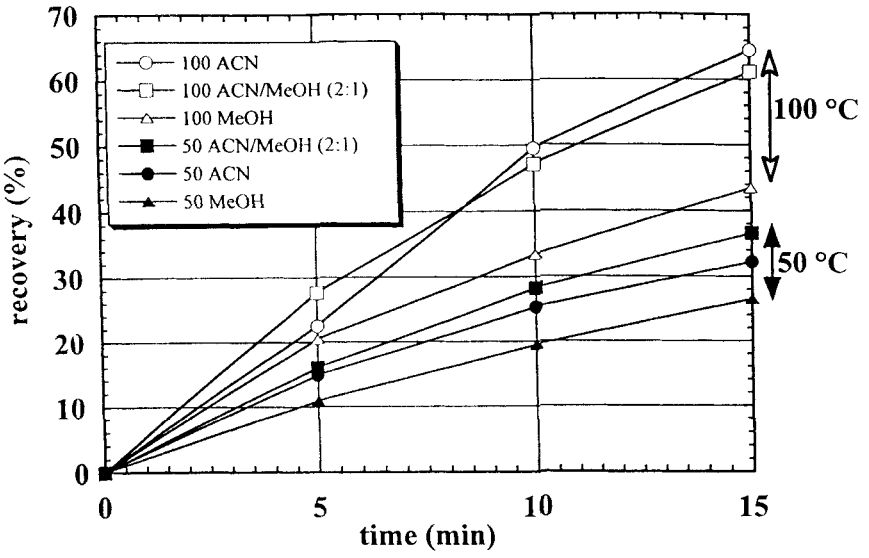


Figure 2. Recovery of felodipine from whole tablets at 1500 psi with different solvents at two different temperatures, 50 °C and 100 °C. Each profile consists of 3 static extractions with a length of 5 minutes each. Flush volume: 60%. Profiles are based on duplicate measurements for each data point.

RESULTS AND DISCUSSION

Extraction of Whole Tablets

In order to minimize the sample pretreament (for single tablet extraction), extraction of a whole tablet was initially attempted. Extraction profiles were obtained by determining the percent recovery of felodipine after each static extraction (3 x 5 minutes). The validated method used for the extraction of felodipine based on ultrasonication utilizes a mixture of acetonitrile and methanol in the proportions 2:1 (v/v). Accordingly, this solvent mixture was investigated as solvent for ASE as well as pure acetonitrile and methanol. As seen in Figure 2, at 50°C, acetonitrile and the mixture of acetonitrile/methanol was found to have the highest recovery (ca 35%) of the three solvent systems.

The low recovery at 50°C for all solvents could be attributed to poor mass transfer of the analyte from the tablet to the extraction solvent. In an effort to improve the mass transfer properties of the solvent, the extraction temperature

was increased from 50°C to 100°C. The recoveries for all solvents increased significantly. Acetonitrile and the acetonitrile/methanol mixture was again the best solvents with a recovery of ca 60%. Although the increased temperature improved the mass transfer properties of the solvents, the extraction was not complete. Consequently, it was concluded that the solvents could diffuse into the tablet and extract the drug.

ASE of Ground Tablet

To increase the extractability of felodipine from formulated tablets, the tablet was ground with a pestle and mortar. The resulting powder was then quantitatively transferred to the extraction cell. Extraction profiles (6 static steps of 10 minutes each) using the ground tablet was constructed (Figure 3A and 3B).

Quantitative extraction was accomplished in 20 minutes with acetonitrile as the extraction solvent at both 50°C and 100°C. The increased surface area of the ground tablet was attributed for the increase in recovery compared to the whole tablet.

Effect of Temperature

Although increased temperatures can improve mass transfer of the extraction solvent, the higher temperature may also cause the drug to degrade. The effect of temperature on decomposition of the drug was tested by monitoring the degradation during ASE at temperatures ranging from 50°C to 200°C (Table 1). The extraction conditions were 1500 psi, acetonitrile, and 15 minutes static extraction.

Only one degradation product (H 152/37) was seen with the ultrasonication method. The degradation product was determined to be 0.11% (RSD 7.3%, n=6) of felodipine based on peak area. The degradation product was quantitatively extracted by ASE at 50°C and 100°C. As the temperature was increased to 150°C and 200°C, the felodipine was observed to degrade.

Specifically, the degradation product peak areas increased from 0.10% at 50°C to 0.26% at 150°C and 0.55% at 200°C. These results show that it is possible to use ASE to determine the felodipine degradation product H 152/37. Since the chromatogram from felodipine tablet extractions did not differ between 50 and 100°C (Figure 4), and no peaks were detected in the placebo extraction it is concluded that temperatures up to 100°C can be used for ASE.

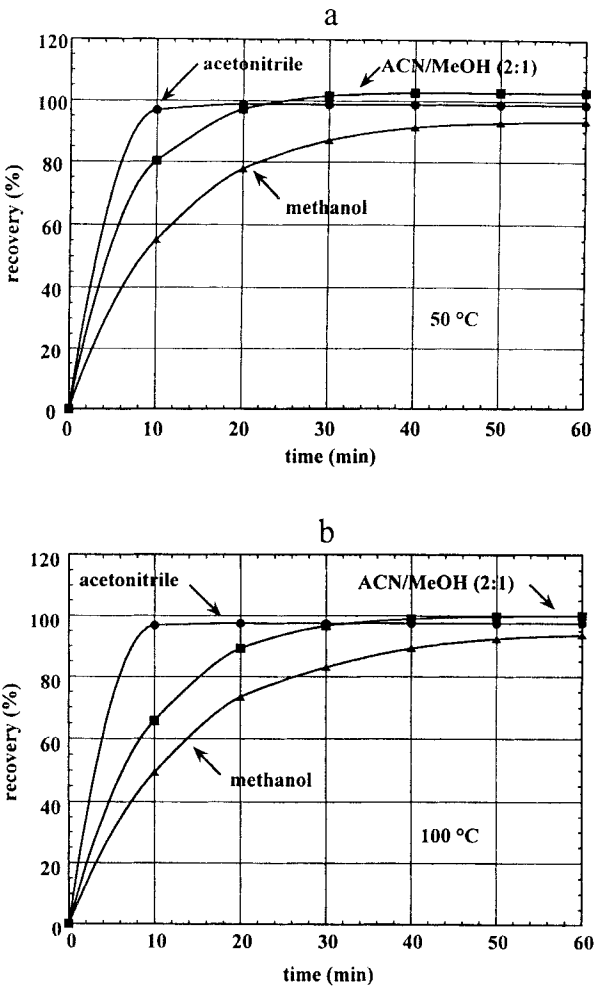


Figure 3. Recovery of felodipine from ground tablets at 1500 psi with different solvents at two different temperatures: A) 50 °C and B) 100 °C. Each profile consists of 6 static extractions with a length of 10 minutes each. Flush volume: 60%. Profiles are based on duplicate measurements for each data point.

Another effect of an increased temperature was that the tablet matrix started to dissolve and was partially transported to the collection vial. This was observed by the presence of particles in the collection vial and discoloration of the extract (Table 1).

Table 1

Degradation Product H 152/37 from Tablets at Four Different Temperatures*

Temperature	Degrading Product 152/37 % of Felodipine (n=3)	RSD (%)	Visual Obseravtion of Collection Vial
50°C	0.10	2.0	Clear solution
100°C	0.11	4.1	Slightly white solution. Particles on bottom.
150°C	0.26	11.8	White/yellow solution. Particles on bottom.
200°C	0.55	8.1	Yellow solution Particles on bottom.

* Extraction conditions: 15 minutes static, acetonitrile, 1500 psi, flush volume 100%

At 200°C almost nothing of the tablet remained in the extraction cell. Furthermore, the peak at the void volume was found to increase with increasing temperature. This peak was thought to be from the coloring agents used in the tablet formulation.

According to Figure 3, acetonitrile is the best solvent giving almost 100% extraction efficiency within a 10 minute long extraction time. Full recovery was achieved at an extraction temperature of 50°C. The occurrence of a colored solution at 100°C and the possibility of a somewhat increased degradation indicates 50°C to be the best temperature of choice.

Final Method

The advantage with extracting whole tablets is that they can be directly added to the extractor without loss of the tablet due to grinding in a mortar. It also eliminates the risk of contamination in connection with transferring of the

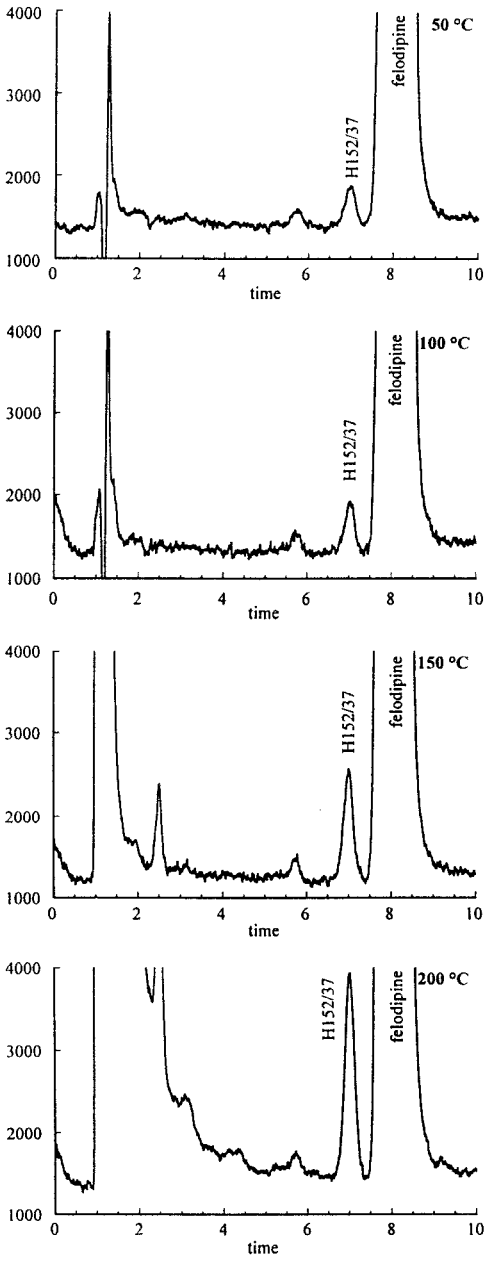


Figure 4. Chromatograms for felodipine and the degradation product H 152/37 at four different temperatures. Extraction conditions: acetonitrile, 1500 psi, flush volume 60%.

ground powder to the extractor cell. An alternate method to grinding the tablet in a mortar is crushing the tablet. Here the tablet was crushed in filter paper which is transferred directly to extraction cell. A pair of flat pliers was used for crushing the tablets. Acetonitrile was used since it was found to be the most efficient extraction solvent. A temperature of 50°C was utilized because higher temperatures were found to dissolve the tablet formulation. Several approaches (A-E) to crushing the tablet were investigated which are illustrated in Figure 5. The experimental parameters and the results obtained are shown in Table 2.

In extractions A, B and C, the tablets were wrapped into filter paper and crushed in a single stroke giving relatively large pieces approximately 1-5 mm in diameter. In A, the cell was filled with hydromatrix, in B no hydromatrix was used, and in C the filter paper was removed giving small losses of tablets that was stuck in the paper. These losses were less than 1% and therefore ignored during this method development procedure. In D and E the tablet was completely crushed inside the filter paper and gently poured on the bottom of the cell. The filter paper containing small pieces of tablet was also put in the cell. This assured a quantitative transfer of the entire tablet.

Comparison of A and B shows that hydromatrix has no significant effect on the extraction efficiency. This means that with hydromatrix in the extraction cell a higher temperature (up to 100°C) might be used to improve the recovery since precipitation in the collection vial then might be avoided. This was confirmed by experiments at 100°C with hydromatrix in the extraction cell. In experiments C-E other ways were investigated to improve the recovery of felodipine. When comparing the setup in C with A and B the largest difference is the distance of the extracting material to the exit of the cell. In C the tablet is located just before the exit and although the recoveries are not significantly higher there is a trend towards both higher recoveries and better precision. Thus a short distance between the extracted material and the exit of the cell seems to be favorable.

Since only 92% recovery was obtained in C with 10 + 10 minute extraction time, it was assumed that the tablet had to be more carefully crushed since 100% recovery was previously achieved within 10-20 minutes with ground tablets. In experiment D, the crushing tool was used on all sides of the filter paper containing the tablet, resulting in a more efficient crushing. The reduction in tablet particle size resulted in recoveries near 100% after a 10 minute extraction. In the final method E, based on result above, the extraction time was increased to 15 minutes and the flush volume of liquid used for transferring the extracted solution from the extractor to the collection vial was increased from 60% (used in experiments A-D) to 100%. The final method gave a felodipine recovery of 98%. Less than 0.5% was found in a second

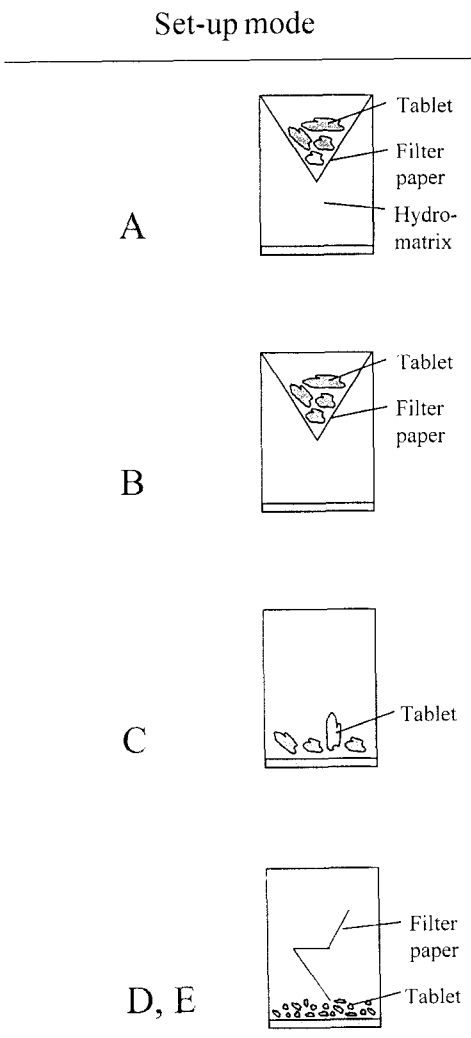


Figure 5. Different approaches for crushing the tablet and transferring the sample to the extraction cell.

extraction step which had a length of 5 minutes. All recoveries were calculated versus a value of 10.17 mg (RSD 0.6%, n=5) determined by the ultrasonication method. There is no difference between ASE and ultrasonication even at the 99% level taking into consideration the RSD values given for the two methods which are 3.8% and 0.6%, respectively.

Table 2

Extraction of Felopipine from Tablets Using Different Sample Pretreatment Techniques*

Set-up mode**	Extraction Time (min)	Recovery (%)	RSD (%)	n	Sample Treatment
A	10	70.9	16.6	10	Tablet crushed to 1-5 mm pieces
	10 + 10	86.4	10.8		Filter paper Hydromatrix
B	10	78.7	14.2	5	Tablet crushed to 1-5 mm pieces
	10 + 10	89.3	9.2		Filter paper
C	10	84.0	6.4	5	Tablet crushed to 1-5 mm pieces
	10 + 10	91.5	4.9		
D	10	95.4	4.1	5	Tablet crushed to <1 mm pieces
	10 + 10	97.6	2.3		Filter paper
E	15	98.4	3.8	10	Tablet crushed to <1 mm pieces
	15 + 5	98.8	3.8		Filter paper

* Extraction conditions: acetonitrile 50°C, 1500 psi, flush volume 60% except E, flush volume 100%.

**For details see Figure 5.

The reason for the lower RSD value in the ultrasonication method is that it involves more tablets. For each experiment 10 tablets are ground in a mortar and from this powder an aliquot of ground material corresponding to a mass of one tablet is taken for the determination of felodipine in a batch. Thereby the inter-tablet variation is compensated for. The ASE value however is determined by extracting ten single tablets. Since there is no difference between the two methods, the ultrasonication step can be directly exchanged with the

ASE step, simply by weighing an amount corresponding to one tablet directly into the extraction cell. The precision in the final analysis method is very good since it is known that the within tablet variation is ca 3-4%. This is equal to the precision value obtained in experiment E in Table 2, which reflects both the within tablet variation and the uncertainty in the final LC analysis. With the developed ASE method it is therefore possible to extract single tablets to determine the between tablet variation. The method can also be used for the determination of felodipine in a batch of tablets.

SAMPLE THROUGHPUT

The throughput is determined by the extraction step, which takes ca 25 minutes per sample. The extraction can be performed automatically with maximum 24 samples processed sequentially in the ASE unit. This means that one extraction sequence of 16 samples can be started in the morning, and 24 samples are loaded for extraction over night. Thus, the throughput is ca 40 samples per day. The manual manipulations are relatively extensive. For the extraction they include weighing of tablets, weighing of the collection vial before and after extraction, crushing of the tablets, and loading of the extraction cells. For the final LC analysis a dilution of the sample with mobile phase is needed to get chromatographic performance. Finally the extraction cells need to be cleaned for the next set of extractions. An overall estimation of all these steps resulted in a man power consumption of 5-6 hours. This time can be reduced with ca 1 hour if an automatic dilutor is used before the LC analysis.

CONCLUSIONS

The development of an automated sample preparation method using ASE is fairly straightforward, especially if analyte solubility data in organic solvents is available. In this particular application extreme temperatures were not needed to obtain high recoveries, thus reducing the risk of target compound degradation.

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