Subcritical Water Extraction and Determination of Nifedipine in Pharmaceutical Formulations

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A rapid and simple continuous method for the extraction of nifedipine from tablets was developed by using pressurized hot water at 150°C. This is the first time that subcritical water was applied to the extraction of low-polarity compounds in pharmaceutical analysis. The method is based on the increment in solubility of nifedipine in subcritical water. Extraction temperature and static and dynamic extraction time were optimized in order to reach quantitative extraction of the drug from the tablets. After extraction, the drug was determined by spectrophotometry by measuring absorbance at 338 nm. Accuracy and precision of the method were determined by analysis of 10 synthetic samples of pharmaceutical formulations prepared with common tablet excipients. Recovery was found to be 99.2% with a relative standard deviation of 1.9%, which indicates that the excipients of the formulation do not interfere in the determination. The method was applied to the determination of the drug content uniformity in tablets.

Analyses of pharmaceutical formulations have been performed using various modern analytical instrumental techniques (1). However, sample preparation always takes place before the analysis itself because it is essential in order to isolate the desired components from the matrixes and because it greatly affects the reliability and accuracy of analysis. In this context, the development of sample preparation procedures plays a very important role in pharmaceutical analysis in order to isolate and to purify the analyte from those matrixes and introduce it into the appropriate instrument.

In conventional methods, the sample is usually first ground in a mortar and then treated with a solvent in which the analytes are extracted with the help of ultrasound; finally, the extracts are centrifuged or filtered. This series of steps is time-consuming.

Since 1999, some modern sample preparation methods have been described (2–6). Kok and Debets (2) developed a new ball mill extraction method for solid dosage forms, which was applied in pharmaceutical analysis. No powdering, weighing, or sonication steps were needed in the sample preparation.

Modern technologies, including the use of new sources of energy, have also been described, such as microwave-assisted extraction (4), subcritical fluid extraction involving CO_2 modified with methanol (5), and accelerated solvent extraction (ASE; 6). The subcritical CO_2 extraction was applied to the determination of lovastatin in tablets, providing recoveries highly dependent on methanol concentration and additive type (5). ASE using methanol was applied for determination of ivermectin from the meat-based formulations (6).

The use of water as a solvent for extraction of low-polarity compounds has been described mainly for environmental purposes. This extraction technique is based on the properties of water provided under subcritical conditions, in which a high temperature and pressure reduces strongly its dielectric constant. In this context, low-polarity compounds of environmental interest, such as anthracene, chrysene, and perylene, each have solubilities about 20 000-fold higher in water at 200°C than at 25°C (7). The strong detriment of the dielectric constant (ε) on water temperature (t) can be estimated by using Equation 1:

$$\varepsilon = 78.54 \times [1 - 4.579 \times 10^{-3} \times (t - 25) + 1.19 \times 10^{-5} \times (t - 25)^2 - 2.8 \times 10^{-8} \times (t - 25)^3] \quad (1)$$

Additionally, under subcritical condition, the viscosity and surface tension of water are also reduced, which makes water an effective solvent for leaching a number of organic compounds that have a broad spectrum of polarity in solid samples (8). However, water does not exhibit toxicity-associated problems like those of organic solvents commonly used in conventional sample preparations. Further, subcritical water conditions can be easily achieved with low-cost laboratory devices.

In the present study, for the first time, the efficiency of subcritical water was assessed for the extraction of a low-polarity drug from pharmaceutical formulations.

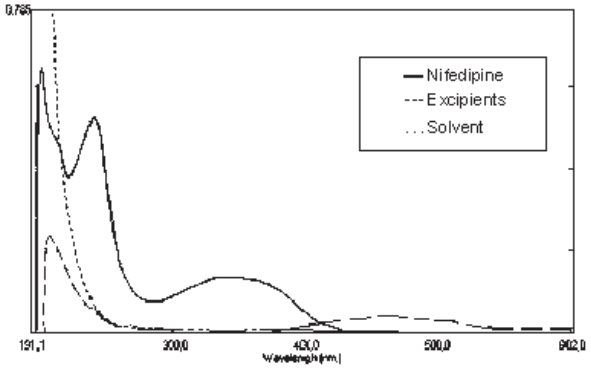

Figure 1. Absorption spectrum of nifedipine overlaid with the spectrum of the excipients and solvent mixture methanol–water (60 + 40, v/v). The excipients/analyte ratio is 10.6:1 (w/w).

Nifedipine was selected as an analyte model; it has an extremely low solubility in water at room temperature but high solubility in methanol. In subcritical conditions, the dielectric constant of water is closer to that observed for methanol. Consequently water under this condition should extract the drug quantitatively from the tablet matrix. After extraction, nifedipine was determined by spectrophotometry.

Nifedipine belongs to a class of medications called calcium channel blockers. By relaxing coronary arteries, nifedipine is useful in treating and preventing chest pain (angina) resulting from coronary artery spasm. Nifedipine has been determined in pharmaceutical formulations by different analytical methods such as differential pulse adsorptive stripping voltammetry (9), solid-phase extraction and high-performance liquid chromatography (HPLC; 10), micellar electrokinetic capillary chromatography (11), and flow injection with polarographic detection (12).

Experimental

Reagents

Deionized water (NANOpure ultrapure water system; Barnstead, Dubuque, IA) was used throughout. Nifedipine (1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine dicarboxylic acid dimethyl ester) reference substance was kindly provided by Laboratorio Chile.

A stock standard solution of nifedipine, 1×10^{-2} M (3463 mg/L), was prepared in methanol (Fisher Chem Alert Guide, HPLC grade) and kept in an amber flask. For calibration purposes, solutions from 2.77 to 41.6 mg/L were prepared in amber flasks [methanol–water (60 + 40, v/v)] by dilution of the stock standard solution.

Instruments and Apparatus

The schematic construction of the extraction unit has been shown elsewhere (8). All tubes (1/16 in., 1/8 in. od) were made from SS 306 stainless steel. Connections were made using Swagelok fittings. The valve used was a Swagelok needle valve SS-ORS2 (5000 psi allowed pressure).

The extraction chamber consisted of a laboratory-made oven (a 28 × 12 × 5 cm aluminium block with controlled temperature). A temperature controller BTC-704 with a thermocouple Type J (operating temperature: 0–400°C) was used to maintain the temperature at the desired value. Inside the chamber, a preheated coil (2 m stainless steel tube SS-316, 1/16 in., 0.1 mm id) was placed to keep the programmed temperature, and it was followed by the extraction cell (a 12 mm id empty HPLC column; Supelco, Bellefonte PA). The deionized water for extraction was pumped using an HPLC pump (Model Series II, Isocratic Pump; Scientific Systems, Inc./LabAlliance, State College, PA). For extraction, the working range of pressure inside the system was kept from 1000 to 2000 psi, which was checked with a manometer (US-Gauge-Bu-2581-AQ; AvCorp International, Charlotte, NC). Nifedipine quantitation was performed using a UV-1603 spectrophotometer (Shimadzu, Columbia, MD).

Pressurized Hot Water Extraction

Tablet formulations were powdered. A quantity of powdered (ca 12 mg) tablet containing nifedipine was accurately weighed and loaded into an extraction cell located inside the aluminium chamber-oven extractor. The oven was coupled to a heating device located on the upper part of the chamber and electronically controlled through a thermocouple to reach 150°C. Water was pumped through the system and, when the extraction cell was filled, flow was stopped for 15 min in order to perform a static extraction of nifedipine from the tablets. After this time, a dynamic extraction was performed by pumping water at a flow rate of 2 mL/min for 20 min. After the pressurized hot water extraction was completed, the nifedipine already in a water phase was quantitatively received in 60 mL methanol contained in a 100 mL-calibrated amber flask. After this process, the extracts were evaluated by ultraviolet (UV) spectrophotometry at 338 nm. Optimization of the extraction considered the following variables: extraction temperature, static and dynamic extraction time, and water flow rate.

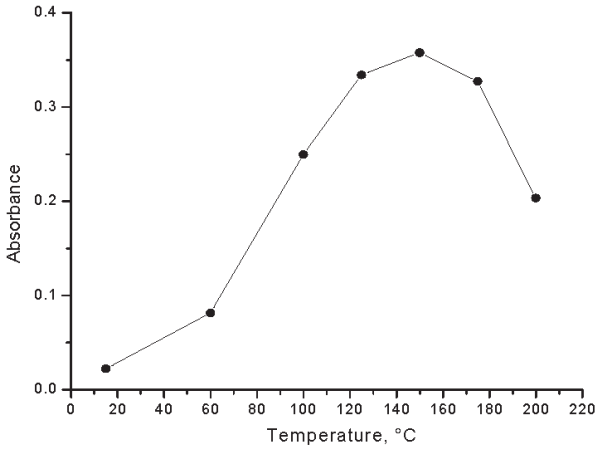

Figure 2. Effect of the temperature of pressurized water on the nifedipine extraction.

Table 1. Recovery of nifedipine from synthetic formulations

Sample	Nifedipine found, mg	Recovery, %
1	9.85	98.5
2	10.23	102.3
3	10.05	100.5
4	9.91	99.1
5	10.09	100.9
6	9.86	98.6
7	9.95	99.5
8	9.53	95.3
9	9.92	99.2
10	9.82	98.2
Mean		99.2
RSD		1.9

Determination of Nifedipine in Synthetic Tablets

A synthetic pharmaceutical formulation containing 10.0 mg nifedipine and 106.6 mg of a mixture of common tablet excipients (magnesium stearate, lactose, starch, talc, iron oxide, and sodium dioctylsulfosuccinate) were mixed. A fraction of powder from 5.00 to 12.00 (± 0.01) mg of synthetic formulation was accurately weighed and transferred into the extraction cell. Then the general procedure was followed.

Determination of Nifedipine in Tablets (Content Uniformity Test)

A total of 10 tablets of nifedipine was weighed. Each tablet was independently powdered, and a fraction of each tablet containing a nominal amount of nifedipine from 0.25 to 1 (± 0.01) mg was accurately weighed, transferred, and treated independently in the extraction cell. Then the general procedure was followed.

Results and Discussion

Optimization of the Extraction Parameters

All variables involved in the extraction process were studied by the univariate method. Tablet formulations were used for optimization studies. Figure 1 shows the absorption spectrum of nifedipine overlaid with the spectrum of the excipient mixture in a weight ratio that is representative of tablet formulations. As can be seen, the selected wavelength of 338 nm provides good selectivity for the analytical measurements.

Effect of Temperature on Nifedipine Extraction

The effect of temperature on nifedipine extraction was checked using different temperatures in the range of 15–200°C. As can be seen in Figure 2, the solubility of nifedipine increases with temperature, tending to quantitative extraction at temperatures near 150°C. Maximum recoveries decreased beyond this value due to the degradation of the nifedipine, which is thermolabile or prone to hydrolytic attack. Taking this effect into consideration, a temperature of 150°C was selected as optimum.

Effect of Time and Flow Rate on Extraction

The extraction efficiency was highly dependent on the static (stopped flow) time. The extraction of nifedipine increased in the 5–20 min range and then remained constant after 20 min. This variable affected extraction significantly, probably because, during static time, subcritical water remains in contact with the sample and increases the dissolution rate of the drug. In previous studies (8, 13), we observed that this variable was not important for the extraction of polycyclic aromatic hydrocarbons and pesticides from soils and airborne particulate matter. Although maximum extraction was reached at 20 min, a static time of 15 min was selected to study the effect of dynamic time.

All previous variables were studied using a constant dynamic time of 15 min. However, it was observed that dynamic time was also an important variable that affected the extraction process. In this context, when dynamic time increased from 10 to 20 min, the extraction efficiencies increased by 40% and remained constant beyond this time.

The flow rate of pressurized hot water was studied between 0.5 and 3.0 mL/min. A linear dependence of extraction efficiency was observed for this variable in the interval 0.5–2.0 mL/min. Beyond this value the signal decreased slightly, and poorer reproducibility was obtained. Consequently, a flow rate of 2.0 mL/min was selected.

On the one hand, a clear advantage of a dynamic extraction process over batch static alternatives like ASE (6) is that, in the first case, the water is cooled outside of the extraction cell, thereby avoiding the possibility of readsorption of the analytes on the solid matrix. In addition, compared with conventional procedures, the proposed extraction method provides the advantage that the extraction and final filtration are performed in one step, eliminating the need of additional manipulation of the sample extract.

On the other hand, despite the advantages provided by subcritical water extraction, it must be stressed that, after the pressurized hot water extraction is done, the nifedipine (in a water phase) is received in 60 mL methanol. Therefore, the advantage of using subcritical water extraction is partially lost.

Analytical Features and Application

The calibration graph was obtained by triplicate evaluation of different concentrations (between 2.7 and 41.6 mg/L) nifedipine in methanol–water (60 + 40). The calibration equation was

$$A = 1.53 \times 10^{-2}\,[\text{nifedipine}] + 6.3 \times 10^{-3} \qquad r = 0.99991 \qquad (2)$$

Table 2. Application of the method in the determination of uniformity of content in nifedipine tablets

Tablet	Nifedipine found, mg[a]	Nifedipine, %
1	9.34	93.4
2	9.60	96.0
3	9.71	97.1
4	8.78	87.8
5	9.53	95.3
6	9.47	94.7
7	9.30	93.3
8	9.48	94.8
9	9.08	90.8
10	9.04	90.4
Mean		93.4
RSD		3.1

[a] Nominal amount 10 mg.

where A is the absorbance and [nifedipine] is the analyte concentration in mg/L.

Accuracy and precision of the method were determined by analysis of 10 synthetic formulation samples according to the procedure given above. The recovery was 99.2% with a relative standard deviation (RSD) of 1.9% (Table 1). These results establish that the excipients normally found in tablets do not interfere with the proposed method. Limit of detection (LOD) and limit of quantitation (LOQ) values were determined considering 3 and 10 signal-to-noise ratios, respectively. LOD and LOQ were 0.28 and 0.94 mg/L, respectively.

The method was applied to the analysis of tablets for uniformity of content. The uniformity of content was assessed by analyzing 10 independent tablets. Table 2 shows the results obtained.

Conclusions

Subcritical water extraction is a simple, fast, and quantitative alternative for extraction of nifedipine from pharmaceutical formulations. Subcritical water extraction is based on a principle similar to ASE (6), but the solvent is water. High temperature and pressure reduce strongly the dielectric constant, viscosity, and surface tension of water, which makes it an effective solvent for leaching low-polarity compounds in solid samples.

An advantage over the ASE method described previously (6) is that the subcritical water extraction method described here does not require a subsequent cleanup step or the need to fill the extraction cell with sand before extraction. Further, the dynamic character of the proposed method allows the water extract to be cooled outside of the extraction cell, thus avoiding the possibility of readsorption of the analytes on the solid matrix.

Acknowledgments

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