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Inverse supercritical extraction of acetaminophen from suppositories

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Abstract

The use of supercritical CO_2 for the isolation of acetaminophen from the non-polar matrix of a suppository is demonstrated. Since acetaminophen is not soluble in pure CO_2 at low pressure, but the waxy matrix is, the later can be extracted, leaving the acetaminophen behind. After studies on acetaminophen solubility as a function of pressure, temperature flow and supercritical fluid volume, optimal extraction conditions were determined. In this method, the matrix is removed using pure CO_2 at 1500 psi and 40°C, and the remaining acetaminophen is then removed from the extraction cell using ultrasound in warm water. This method is an alternative to the US Pharmacopoeia's (USP) method for this kind of formulation, which involves the dissolution of the matrix in hexane with the subsequent liquid–liquid extraction of the acetaminophen into water.

Keywords: Inverse supercritical extraction; Acetaminophen suppositories; Matrix removal

1. Introduction

Traditionally supercritical fluids have been used to extract traces of non-polar or moderately-polar compounds from their matrices. Nevertheless, in some cases, the reverse is required. This is the case for non-polar pharmaceutical formulations such as ointments, creams and suppositories. For these analyses, the goal is to remove the nonpolar matrix while retaining the active drug (usually a polar compound). The normal procedure for these analyses involves three steps: the dissolution of the non-polar matrix using hexane or other appropriate organic solvent, the subsequent extraction of the active drug using liquid–liquid extraction or solid phase extraction, and the qualification and quantification of the analyse via reverse-phase HPLC.

Recently, a new approach named 'inverse supercritical extraction' was proposed for these formulations. Here the supercritical fluid is used to remove the non-polar matrix, leaving the polar drug behind. This approach was demonstrated for the analysis of Zovirax ointment 5% [1] and for the analysis of Neosporin ointment and cream [2].

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In this paper we present a new method for the analysis of suppositories using supercritical CO_2, as the solvent for the removal of the waxy matrix. Acetaminophen suppositories were used as a model for this method. The acetaminophen is quantitatively retained in the extraction cell and then it is extracted with warm water (~ 50°C) using an ultrasonic probe. The acetaminophen is quantified via HPLC using the conditions suggested by the US Pharmacopoeia (USP) [3].

2. Experimental

Pediatric acetaminophen suppositories (120 mg labeled strength) were purchased from a local drugstore. All extractions were performed on a SFX 2-10 supercritical fluid extractor attached to a model 260-D syringe pump (ISCO, Lincoln, NE), using 10 ml stainless steel extraction cells. A paper filter cut from a Soxhlet thimble was used as a pre-filter over the cell frit. Commercial grade CO_2 (General Gases, San Juan, PR) was used during this research. The restrictor consisted of a stainless steel capillary tubing, 0.25 mm i.d. and 32 cm long. The flow was controlled manually using the extractor valve. To avoid restrictor plugging, the restrictor was heated all the time to about 75°C.

The HPLC method was based on the assay procedure recommended in the USP official monograph for acetaminophen capsules [3]. The HPLC system was a Hewlett-Packard model 1050 equipped with a variable wavelength detector and a 10 μl injection loop. The column was a Supelcosil LC-18-DB, 250×4.6 mm (Supelco, Belofonte, PA). The mobile phase was water–methanol (3:1 v/v). The detection was performed at $\lambda = 243$ nm. The injection concentration was about 12 μg ml^{-1}. Total running time was approximately 6 min per sample and the acetaminophen retention time was about 3.5 min. This procedure was used to analyze both the SFE extract and the USP extract.

The current USP method for the analysis of acetaminophen suppositories involves the dissolution of the suppository in 30 ml hexane, with the subsequent liquid–liquid extraction using four 30 ml portions of water. All aqueous portions are combined in a 200 ml volumetric flask and diluted to volume using a mobile phase. This extract was further diluted 100-fold.

3. Results and discussion

A requirement for a successful inverse supercritical extraction is that the analyte be insoluble in the supercritical fluid. The acetaminophen solubility was studied as a function of CO_2 pressure, flow and temperature. For the temperature experiments, this was varied from 40 to 120°C. The pressure was set at 1500 psi and the flow was set at about 2 ml min^{-1}. In another set of experiments the pressure was varied from 1500 to 5000 psi, the temperature was set to 40°C and the flow was 2 ml min^{-1}. The effect of flow was evaluated from 0.5–6 ml min^{-1} at 40°C and 1500 psi. During all these experiments, approximately 100 mg of acetaminophen USP raw material were accurately weighed, placed into the extraction cell and subjected to the different conditions. The cell was pressurized and the acetaminophen was extracted during 5 min in the static mode. Then, the extraction valve was opened and 30 ml CO_2 were passed through the cell and collected in 50 ml of the mobile phase. After each experiment, the cell was placed in a desiccator until it reached ambient temperature and then weighed. The collection solvent was analyzed for acetaminophen using the USP method. Tables 1–3 shows the results of these experiments. Under all conditions studied, the loss of acetaminophen was less than 0.02% of the sample.

Based on the results of the solubility experiments, the extraction conditions were set at 40°C, 1500 psi (103 bar) and the flow was set to about 2 ml min^{-1}. This temperature was chosen since it is above the CO_2 critical temperature and yet low enough to avoid fast melting of the suppository. This is an important consideration since the acetaminophen could be lost via physical entrainment or mechanical transfer through or around the cell frits if the matrix becomes too fluid as described by Moore and Taylor [2] in their paper on Neosporin ointment and cream. All extraction

Table 1
Solubility of acetaminophen as function of pressure

Pressure (psi)	Percent loss (as function of starting weight)				
	Trial 1	Trial 2	Trial 3	Mean	S.D.
1500	0.006	0.010	0.008	0.008	0.0020
2500	0.000	0.017	0.003	0.007	0.0091
3500	0.009	0.008	0.004	0.007	0.0026
4500	0.006	0.011	0.006	0.008	0.0029
5500	0.013	0.012	0.012	0.012	0.0006

Approximately 100 mg acetaminophen USP raw material were accurately weighed and placed into the extraction cell. The cell was pressurized and the acetaminophen was extracted during 5 min in the static mode. Then the extraction valve was opened and 30 ml CO_2 was passed through the cell and collected in 50 ml of the mobile phase. The acetaminophen concentration in the collection solvent was determined by HPLC using the USP method.

experiments described hereafter were performed using these conditions unless stated otherwise.

The matrix-removal efficiency of the USP method was evaluated by placing the suppository into a 50 ml centrifuge tube. After weighing the tube with the suppository, 30 ml hexane was added. The tube was capped, shaken during 5 min and centrifuged at 1000 rpm for 10 min. The non-soluble acetaminophen and other excipients formed a pellet at the bottom of the tube. The hexane layer was carefully decanted and the tube was allowed to dry, covered with a tissue paper, in a hood. The next day the tube was weighed and the weight difference of the extracted matrix was calculated. The mean extracted matrix reported as the percent of extracted matrix was 90% with a S.D. of 1.3% ($n = 6$).

The extraction profile for the suppository wax (Fig. 1) agreed with the theoretical extraction profile of an analyte extracted with a dynamic system [4]. This profile shows that under the extraction conditions used, the first 20 ml CO_2 passed through the cell extracted about 80% of the matrix and further extraction increases the amount of matrix extracted close to 90%. Although, the 10% increase in the matrix extraction seems to be small when compared with the increase in time and CO_2 volume, it implies that the sample injected is cleaner and this improves the peak shape and column life. Moreover this extraction efficiency matches the one obtained by dissolving the suppositories in hexane. Based on this

Table 2
Solubility of acetaminophen as function of temperature

Temperature (°C)	Trial 1	Trial 2	Mean	S.D.
40	0.001	0.001	0.001	0.0000
60	0.001	0.001	0.001	0.0000
80	0.002	0.002	0.002	0.0000
100	0.002	0.003	0.003	0.0007
120	0.006	0.003	0.005	0.0021

Approximately 100 mg acetaminophen USP raw material were accurately weighed and placed into the extraction cell. The cell was pressurized and the acetaminophen was extracted during 5 min in the static mode. Then the extraction valve was opened and 30 ml CO_2 was passed through the cell and collected in 50 ml of the mobile phase. The acetaminophen concentration in the collection solvent was determined by HPLC using the USP method.

Table 3
Solubility of acetaminophen as function of flow

Flow (ml min^{-1})	Trial 1
0.5	0.000
1.4	0.002
2.9	0.001
4.2	0.000
5.3	0.006

Approximately 100 mg acetaminophen USP raw material were accurately weighed and placed into the extraction cell. The cell was pressurized and the acetaminophen was extracted during 5 min in the static mode. Then the extraction valve was opened and 30 ml CO_2 was passed through the cell and collected in 50 ml of the mobile phase. The acetaminophen concentration in the collection solvent was determined by HPLC using the USP method.

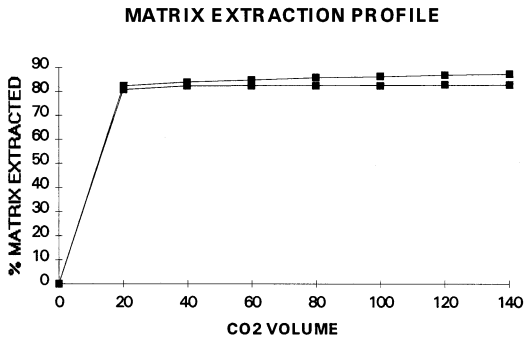

Fig. 1. Matrix extraction profile for the acetaminophen suppositories. The extraction conditions were 40°C, 1500 psi (103 bar) and the flow was set to about 2 ml min^{-1}. The supercritical CO_2 was de-pressurized into glass-beds filled vials that were accurately weighted after the desired volume passed through.

data we chose a volume of 130 ml CO_2 as the extraction volume.

A combination of static–dynamic and only dynamic extraction were evaluated during this study. In the static–dynamic experiments, the cell was pressurized and held in the static mode for a given time and then the extraction was continued in the dynamic mode. The CO_2 was depressurized into 50 ml of the mobile phase in order to quantify the amount of acetaminophen loss during the clean-up process. Using a static period of 20 min, then continued in the dynamic mode until 47 ml were passed through the cell, the amount of acetaminophen loss was 2% of the label strength (LS). When the static period was reduced to 5 min, the amount of acetaminophen loss was reduced to 0.5% LS, even when the volume of CO_2 that passed through the cell was increased 3-fold (146 ml). We attribute this loss to the mechanical transfer of acetaminophen particles through the pores of the cell frit due to the higher fluidity of the matrix after 20 min in the fluid. To confirm this hypothesis, another set of experiments were performed where either glass beads or silica were added to the bottom of the cell in order to increase the retention of the analyte while permitting the removal of the matrix. The average amount of acetaminophen loss during these experiment was 0.2% LS with an average amount of CO_2 used of 112 ml per extraction. Since these results were similar to those obtained using a 5 min static period, it was decided not to add the silica and use the 5 min static period instead.

Our first trial to remove the acetaminophen from the cell was using methanol-modified CO_2 after the matrix removal. Nevertheless, after several sets of experiments, the amount of acetaminophen extracted was between 80 and 90% LS, so the strategy was changed. The other approach was to extract the acetaminophen into a mobile phase using ultrasound. After the matrix removal was completed, the cell was disassembled, transferred to a 100 ml glass beaker and rinsed with 20 ml of the mobile phase. Another 60 ml were added to the beaker to make the final volume about 80 ml. Then the beaker was placed under a ultrasonic probe (Sonicator Ultrasonic Processor, Model XL, Heat Systems, Fanningdale, NY) and extracted during 15 min. The mobile phase was transferred to a 100 ml volumetric flask and the volume completed with the mobile phase. This extract was further diluted 100-fold.

Using the previously described conditions (5 min static; 140 ml dynamic; 40°C; flow 2 ml min^{-1}; 1500 psi) to remove the matrix and extract the acetaminophen into the mobile phase with ultrasound, the extraction efficiency was compared against the USP method. A t-test demonstrated that the amount of acetaminophen determined with our method was significantly less (USP = 111% LS; SFE 104% LS; $n = 6$; $P > 0.5$) than the amount determined using the standard method.

A dynamic extraction was then tested where the suppository was placed into the cell and the extraction begun as soon as the cell reached the desired pressure (1500 psi). Flow, temperature and the amount of CO_2 used were the same as before. The acetaminophen was extracted into warm water (50°C) instead of the mobile phase. Total time to complete the extraction was about 1.5 h (about 1 h for the clean-up step and 30 min to complete the ultrasonic extraction). Using these conditions, no significant difference was found in the amount of acetaminophen determined with the two methods ($n = 5$). Table 4 shows the results and the t-test for this experiment.

Table 4
Comparison between methods

% LS		*t*-Test between USP and SFE data		
USP	SFE		USP	SFE
109.1	108.7	Mean	108.7	109.0
109.1	108.9	Variance	0.36	0.48
109.1	108.1	Observations	5	5
108.3	109.6	Pooled variance	0.42	
107.8	109.8	Hypothesized mean Difference	0	
		Degrees of freedom	8	
		t	−0.830	
		$P(T<=t)$ two-tail	0.431	
		t Critical two-tail	2.306	

Acetaminophen concentration (% LS) determined after the individual extraction of 10 suppositories from the same lot. Five were extracted according to the USP monograph but instead of a composite, individuals were analyzed. The other five were analyzed using the method developed. LS = 120 mg. A *t*-test demonstrated no significant differences between the methods.

4. Conclusions

The use of inverse supercritical extraction as a method for the clean-up of acetaminophen suppositories prior to the determination of the active drug was demonstrated. Under the proposed conditions, this method generates equivalent results when compared to the USP method. This method is faster than the USP method since the latter suffers from the formation of an emulsion that require about 8 h to separate for each of the four extractions. Moreover, the use of inexpensive commercial grade CO_2 instead of the more expensive SFC/SFE grade CO_2, reduces the analysis cost.

Loss of the active drug during the matrix-removal step seems to be related to physical entrainment of the analyte as the matrix liquefies due to high temperature or contact with the supercritical fluid during an extended static extraction period. Using lower temperatures, a shorter static extraction period, or a dynamic extraction seem to overcome this problem.

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