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Focused microwave-assisted extraction and LC determination of the active ingredient in naproxen-based suppositories

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Abstract

A simple and rapid open-vessel focused microwave-assisted extraction (FMAE) method followed by LC analysis was developed for the determination of naproxen in suppositories.

Parameters which might affect the FMAE method, such as nature and volume of the extraction solvent, temperature and extraction time were optimized.

The extraction solvent consisted of methanol/sodium hydrogen carbonate (pH 8.7; 0.1 M) (50:50, v/v). Extractions were performed by reaching the target temperature of 70 °C in a 7 min linear ramp and then maintaining the target temperature for 3 min. Chromatographic analysis was performed on a Discovery RP-Amide C16 column (250 mm × 4.6 mm i.d., 5 μm particle size). The mobile phase consisted of acetonitrile/potassium dihydrogenphosphate (pH 3.0; 25 mM) (40:60, v/v).

The complete analytical procedure was validated with regard to limit of quantification, linearity, precision and accuracy.

The advantages of the proposed method in comparison to conventional methods are decreased extraction time, reduced solvent consumption and no further sample clean-up steps required before liquid chromatographic analysis.

Keywords: Microwave-assisted extraction; Reversed-phase chromatography; Suppositories; Naproxen

1. Introduction

Sample preparation is often the most complex and time-consuming step of analytical procedures. In pharmaceutical analysis non-polar formulations such as suppositories are among those which require quite long and solvent consuming procedures for active ingredient extraction. This is due to the necessity of removing the non-polar matrix while retaining the active ingredient. Conventional methods for active ingredient extraction from this type of formulations generally involve several steps such as melting, dispersion of the matrix in the appropriate hot organic solvent, cooling, centrifuging and/or filtering.

Since 1985 many applications of microwave heating for the extraction of compounds from sample matrices have been developed.

Microwave assisted extraction (MAE) is the process of using microwave energy to heat solvents in contact with a sample to partition analytes from the matrix into the solvent itself. Heating is obtained owing to the direct effect of microwaves on molecules by ionic conduction and dipole rotation [1–4].

Two types of microwave heating systems, commonly referred as closed- and open-vessel systems, are commercially available for the analytical laboratory. In the first case the sample is immersed in a microwave-absorbing solvent in a high-pressure closed container and irradiated with microwave energy which causes the solvent to be heated well above its boiling point. This system is generally named pressurized MAE (PMAE). In the latter system the sample is placed in an open vessel, irradiated by a focused microwave radiation with the solvent being refluxed at atmospheric pressure till the completion of extraction. This technique is named focused MAE (FMAE) [3–5].

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One of the main advantages of MAE is the reduction of extraction time due to the difference in heating performance between microwave radiation and conventional heating. In the latter case a finite period of time is needed to heat the vessel before the heat is transferred to the solution, while microwaves heat the solution directly [1–4].

Additionally a significant reduction in organic solvent consumption can be achieved by replacing traditional pre-treatment procedures with microwave-assisted extraction.

These undoubted advantages have made MAE an attractive alternative to conventional techniques and a number of works have been published in the last 20 years about this modern sample treatment method [1,2,5,6].

MAE technique, especially in the closed-vessel moiety, has been successfully used in the extraction of a variety of analytes, both organic and inorganic, from environmental matrices [7–14] as well as from food [15–18] and medicinal plants [19–22]. A method for pre-treatment of liquid nutritional supplements for determination of selenium concentration has also been reported [23]. The determination of sunscreen agents in cosmetic products using microwave-assisted extraction has been described by Shih and Cheng [24].

In the pharmaceutical field very few papers have been published dealing with microwave technology applied to sample preparation. In the work of Sparr Eskilsson et al. a method for microwave assisted extraction of felodipine and one of its degradation products from tablets is described [25].

To our knowledge no papers describing extraction of the active ingredient from suppositories by microwave technology have been published.

Among non-conventional methods for suppositories treatment before active ingredient chromatographic determination the use of supercritical CO_2 for the isolation of acetaminophen from suppositories has been described [26].

The aim of this work was to apply FMAE to the extraction of the active ingredient from suppositories in order to shorten and simplify the sample preparation step and reduce organic solvent consumption in comparison to traditional compendial methods. This novel sample preparation technique, followed by a validated LC method, is also proposed as an alternative strategy for large-scale screening of suppository dosage forms aimed to the detection of counterfeit and substandard drugs.

Naproxen suppositories were used as model pharmaceutical formulations for this study.

2. Experimental

2.1. Chemicals

Naproxen reference standard was received from Sigma Chemical Company (St. Louis, MO, USA). Naproxen-based suppositories were obtained from the national market (labeled composition: naproxen 500 mg; excipients: semi-synthetic glycerides). Potassium dihydrogen phosphate and sodium hydrogen carbonate were from Fluka Chemie AG (Buchs, Switzerland). HPLC-grade methanol and acetonitrile were from Sigma–Aldrich (Steinthem, Germany); HPLC-grade water was supplied by BDH (Poole, UK). All other reagents were of analytical grade.

2.2. Microwave-assisted extraction

A FMAE unit (Star System 2, CEM, Matthews, NC, USA) equipped with a 2450 MHz magnetron was used for extraction of the active ingredient from suppositories.

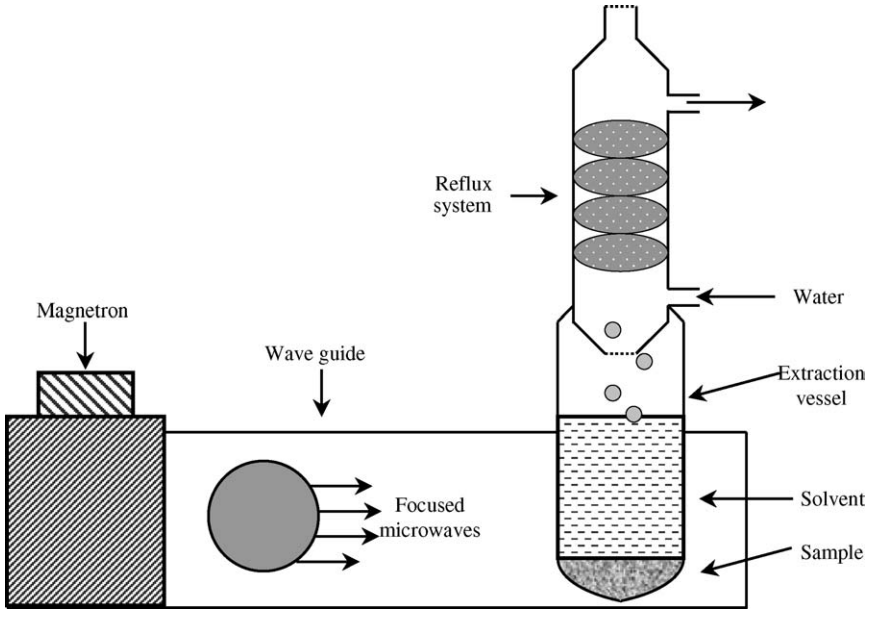

Fig. 1. Schematic diagram of FMAE apparatus.

One suppository was weighed, placed into a 250 ml quartz vessel and 50 ml of the extraction mixture consisting of methanol/sodium hydrogen carbonate 0.1 M (50:50, v/v) was added.

The vessel was placed into the microwave irradiation cavity and fitted to the condenser. The whole system was open and run at atmospheric pressure. A schematic diagram of the apparatus is shown in Fig. 1 [3].

Extractions were performed by reaching the target temperature of 70 °C in a 7 min linear ramp and then maintaining it for 3 min.

After the completion of the extraction procedure the vessel was drawn out from the cavity and cooled down to room temperature. An aliquot of the liquid below the surface of the extraction medium where the wax matrix was lying was then withdrawn to obtain sample solutions.

2.3. Chromatographic analysis

The chromatographic apparatus consisted of an HPLC system Series 1050 equipped with an automatic injector and a diode array detector Series 1040M (Agilent Technologies Deutschland GmbH, Waldbronn, Germany). For data collection and calculation Chemstation software was used (Agilent Technologies).

The chromatographic column was a Discovery RP-Amide C16, 250 mm × 4.6 mm i.d., 5 μm particle size (Supelco, Bellefonte, PA), thermostated at 30 °C.

The mobile phase consisted of acetonitrile/potassium dihydrogenphosphate (pH 3.0; 25 mM) (40:60, v/v), delivered at a constant flow rate of 1.3 ml min^{-1}. The monitor wavelength was 230 nm and the injection volume was 10 μl.

2.4. Standard and sample solutions

Standard stock solutions of naproxen at a concentration of about 2 mg ml^{-1} were prepared by dissolving the proper amount of reference substance in the mobile phase and stored at +4 °C for a week at most.

The standard working solutions at the concentration of the calibration range were prepared by appropriate dilution of the stock solution with the mobile phase immediately before the chromatographic analysis.

Sample solutions were obtained by 50-fold dilution of the extracts with the mobile phase and injected immediately after preparation.

3. Results and discussion

3.1. Extraction method development and optimization

With the aim of testing the application of microwave technology to the development of an alternative method for active ingredient extraction from suppositories, both techniques, PMAE and FMAE, were considered.

After an extensive literature survey the latter was chosen since it was considered to be more suitable to the intended pharmaceutical application. As a matter of fact, the milder extraction conditions of FMAE systems, due to the focused nature of the microwave energy that avoids the application of high power, seemed to be more appropriate to pharmaceutical sample treatment [5].

Furthermore open-vessel systems offer increased safety of sample handling and the possibility to extract larger sample sizes compared to extractions in pressurized closed vessels [1,3,5].

Finally for the chosen system a moderate investment, lower than for PMAE, is required.

The FMAE system used in this work incorporates a single magnetron to generate microwave energy: the microwaves are directed through a waveguide which has two cavities positioned along its length. Each cavity has its own microwave slot control: when the slot is open microwaves enter the cavity; when the slot is closed microwaves are shut off. Infrared sensors measure the temperature in each cavity and, based on a feedback system, energy supplied to each vessel is adjusted. A commercial unit that has six reaction vessels is also available.

The first stage of method development concerned the choice of the extraction solvent.

As with other techniques the solvent or the solvent mixture should efficiently solubilize the analyte of interest without significantly extracting matrix materials and should be compatible with the following analytical steps.

When selecting a solvent for MAE, the microwave absorbing properties of the system must also be carefully considered. Since the wax matrix of suppositories is not expected to absorb microwave radiation, a microwave absorbing solvent should be chosen in order to obtain heating and hence effective extraction.

A common starting point for MAE solvent choice is using the same solvent as prescribed for the traditional extraction.

In British Pharmacopoeia [27] a method is described where methanol is used to extract naproxen from suppositories so this solvent, that additionally has good microwave absorbing properties, was used for preliminary experiments.

First tests were carried out placing one suppository into the vessel with 100 ml of methanol and setting extraction temperature at 40 °C. This temperature was reached by a 6 min linear ramp and then maintained for 15 min.

In the above-cited conditions extraction efficiencies not greater than 57% of labeled strength were obtained.

By raising the target temperature to 50 °C the recoveries increased to values varying from 74 to 86% with an average extraction efficiency of 81%.

At this stage of method development it was also observed that similar recoveries were obtained reducing solvent volume to 50 ml.

It was not possible to further increase the temperature since approaching methanol boiling point (65 °C) irregular

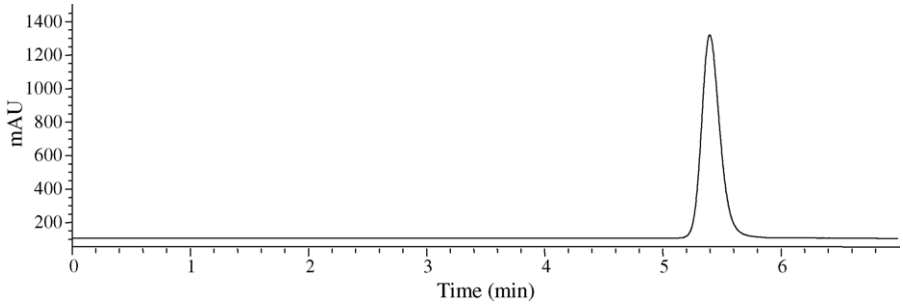

Fig. 2. Representative chromatogram of a sample solution obtained by 50-fold dilution of the extract with the mobile phase (naproxen concentration 0.2 mg ml^{-1}). Column: Discovery RP-Amide C16, 250 mm × 4.6 mm i.d., 5 μm. Mobile phase: acetonitrile/potassium dihydrogenphosphate (pH 3.0; 25 mM) (40:60, v/v) at a flow rate of 1.3 ml min^{-1}. UV detection at 230 nm. Injection volume: 10 μl.

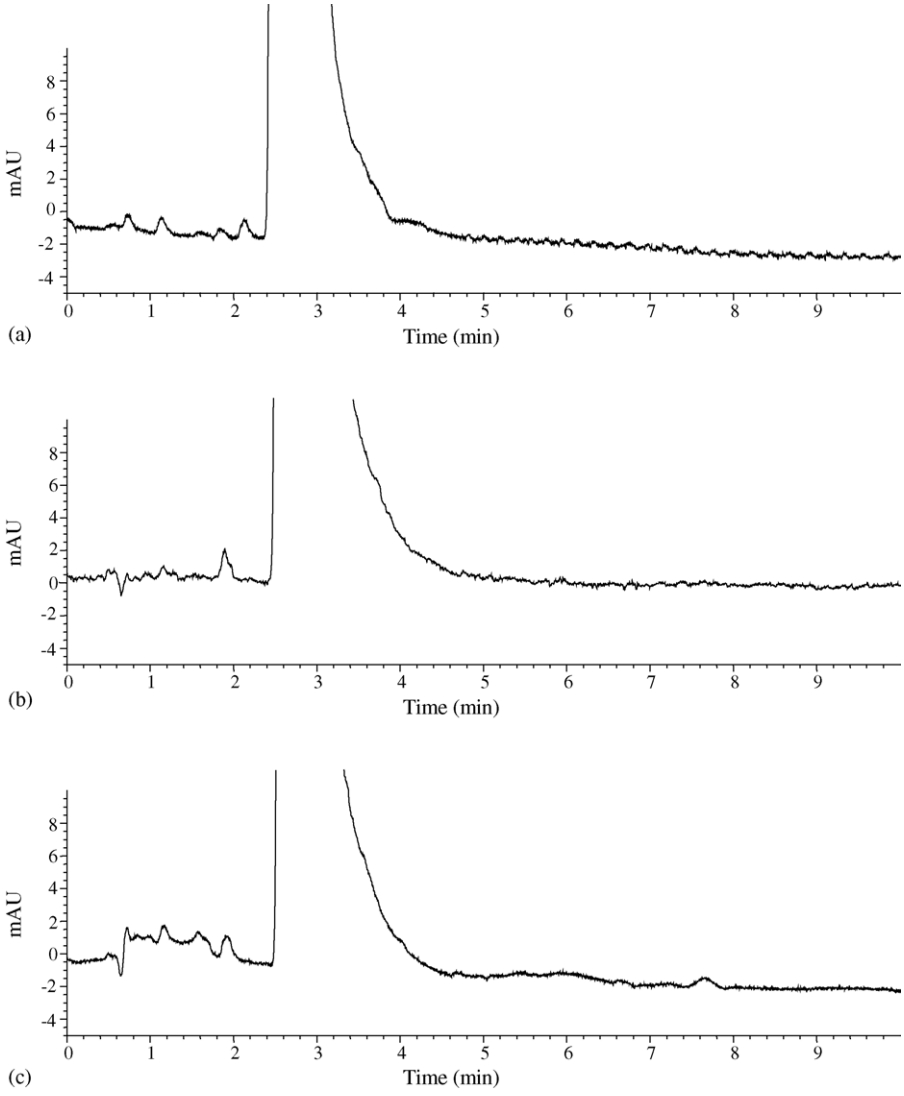

Fig. 3. Chromatograms of (a) naproxen bulk drug solution; (b) FMAE sample solution; (c) BP extraction method sample solution. Naproxen concentration 0.6 mg ml^{-1}. Column: Zorbax C18, 100 mm × 4.0 mm i.d., Mobile phase: acetonitrile/potassium dihydrogenphosphate (pH 2.0; 10 mM); (50:50, v/v) at a flow rate of 1.5 ml min^{-1}. UV detection at 230 nm. Injection volume: 20 μl.

boiling took place thus causing dispersion of suppository fragments out of the vessel.

Ethanol, which has a higher boiling point, was so investigated as an alternative extraction solvent.

The experiment was carried out at 60 °C but, in spite of a good solubility of naproxen in ethanol, not more than 10% of the expected concentration of the active ingredient was recovered in the extraction medium.

A methanol/water 80:20 (v/v) mixture that allowed to reach extraction temperatures up to 60 °C was also tried but still in this case very low recoveries were achieved.

Because of the presence in naproxen molecular structure of a carboxylic acid functional group which makes it freely soluble in alkaline aqueous media, an experiment was performed by replacing the methanol/water mixture with methanol/0.1 M sodium hydrogen carbonate (pH 8.7).

A 50:50 ratio of the two solvents showed to be effective to obtain quantitative recoveries of the active ingredient. Extraction target temperature was set at 70 °C with an overall procedure time of 22 min.

Optimization of the developed extraction method was then obtained by reducing sample treatment time to 10 min as described under Experimental.

A simple and rapid reversed-phase liquid chromatographic method was then developed for naproxen determination in sample solutions obtained by dilution of the extracts. A representative chromatogram is shown in Fig. 2.

In order to verify that during FMAE no degradation of the active ingredient occurred extraction samples were analysed with a stability indicating validated LC method [28].

The chromatographic profile was compared to those of samples obtained by BP extraction method and to naproxen bulk drug solutions. The comparison showed no significant increase in the pre-existing impurities and no evidence of new induced degradation products in samples obtained by FMAE. Chromatograms are shown in Fig. 3.

3.2. Validation of the analytical procedure

The complete analytical procedure, consisting of two steps, FMAE extraction and reverse phase LC assay, was then validated with regard to limit of quantitation, linearity, precision and accuracy.

Table 1
Precision data: intra- and inter-day repeatability

	Day 1	Day 2	Day 3	Overall
n	6	6	6	3
R.S.D. (%)	1.13	3.12	2.90	0.97

n = number of determinations.

3.2.1. Limit of quantitation and linearity of the chromatographic method

The minimum concentration at which the analyte could be reliably quantified, based on a signal-to-noise ratio of 10:1 was 0.19 μg ml^{-1}.

Linearity of the assay was examined by injection of naproxen standard solutions at five concentration levels in the range 0.16–0.24 mg ml^{-1}. Each concentration was tested in triplicate. The calibration curve obtained by plotting the naproxen peak area against the concentration of standard solutions was linear in the above mentioned concentration range. The regression equation was $y = 6.09 \times 10^4 x - 142.2$, $r^2 = 0.999$. The standard error of slope and intercept were 1.08×10^3 and 219.3, respectively.

3.2.2. Precision and accuracy

Method precision, expressed as inter- and intra-day repeatability was determined by replicate analyses of single suppositories of known weight. The results are shown in Table 1.

Accuracy of the method was determined by comparison of the results of the proposed analytical procedure with those of BP [27] method. Here 10 suppositories are dispersed in 500 ml of methanol on a water bath for 40 min with the aid of ultrasounds. After cooling at 5 °C for 1 h the solution is centrifuged and the filtered supernatant liquid is used for chromatographic analysis.

Ten suppositories were analysed using the method developed and 10 were extracted according to the BP monograph. Mean recoveries, S.D. and R.S.D.% were calculated for both

Table 2
Accuracy data

mg/suppository (% of label claim)		*t*-Test between BP and FMAE data		
BP	FMAE		BP	FMAE
527.3 (105.5)	541.2 (108.2)	Mean	543.2	534.9
440.4 (88.1)	511.0 (102.2)	Standard deviation	56.4	12.9
600.3 (120.1)	544.7 (109.0)	R.S.D.%	10.4	2.4
523.6 (104.7)	516.7 (103.3)	Observations	10	10
576.3 (115.3)	540.5 (108.1)	Pooled standard deviation	40.9	
481.5 (96.3)	546.5 (109.3)	Degrees of freedom	18	
593.8 (118.8)	538.2 (107.6)	*t*	0.45	
585.1 (117.0)	539.6 (107.9)	*t* critical ($\alpha = 0.05$)	2.10	
600.8 (120.2)	523.7 (106.5)			
503.0 (100.6)	547.0 (109.4)			

methods. A *t*-test at the 95% confidence level demonstrated no significant differences between the two methods. Results are shown in Table 2.

3.3. Multi-suppository extraction

The method described has been applied to single suppositories analysis thus giving a value of within suppository variation of active ingredient in a batch, but it could be extended, with some modifications, towards determining the average naproxen content of a batch.

With this aim some experiments have been performed. Preliminary tests demonstrated that it is possible to extract 10 units at a time, divided in two vessels, by increasing the extraction volume to 150 ml per each vessel. All other experimental conditions were unchanged. The mean recovery of naproxen was 104.7% of suppositories labeled strength (R.S.D.% = 2.5, $n = 3$).

4. Conclusions

In this work the use of FMAE as a technique for naproxen suppositories sample preparation has been described. The method developed generates equivalent results when compared to conventional BP method.

The major benefits of this technique in comparison to the conventional method are decreased extraction step time, reduced solvent consumption and no further sample clean-up steps required before liquid chromatographic analysis.

The analytical procedure described could provide an alternative fast and accurate method for rapid screening of naproxen-based suppositories aimed to the detection of counterfeit and substandard drugs.

It seems possible to extend this methodological approach towards other non-polar matrix formulations by opportunely varying extraction conditions.

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