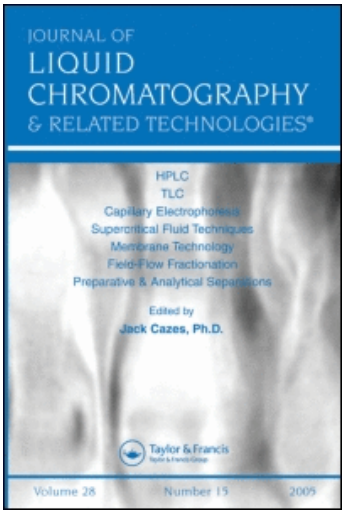

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DETERMINATION OF AMFEPRAMONE HYDROCHLORIDE, FENPROPOREX, AND DIAZEPAM IN SO-CALLED "NATURAL" CAPSULES USED IN THE TREATMENT OF OBESITY

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DETERMINATION OF AMFEPRAMONE HYDROCHLORIDE, FENPROPOREX, AND DIAZEPAM IN SO-CALLED "NATURAL" CAPSULES USED IN THE TREATMENT OF OBESITY

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ABSTRACT

A simple and rapid method was developed for the determination of amfepramone hydrochloride, fenprorex, and diazepam in capsules using high performance liquid chromatography (HPLC) with UV detection. This procedure provided conditions for the separation of the active ingredient from the complex matrices of the dosage forms by extraction in methanol. Isocratic reversed phase chromatography was performed using acetonitrile, methanol, and aqueous 0,1% ammonium carbonate (50:10:40) as a mobile phase, LiChrospher 100 RP 18 column (125 x 5 mm id, 5μm), a column temperature of 25 ± 1°C and detection at 230 nm.

The calibration curves were linear over a wide concentration range (20-2000 $\mu g.mL^{-1}$ to amfepramone hydrochloride, 8-800 $\mu g.mL^{-1}$ to fenproporex, and 4-200 $\mu g.mL^{-1}$ to diazepam) and good analytical recovery (87.1 to 107.8%) was obtained. The method is accurate and precise, as well as having advantages such as simplicity and short duration of analysis. Twenty samples of pharmaceutical preparations labelled as "natural" products were

analysed. Anorectics and diazepam, were detected in 40% of the samples.

INTRODUCTION

The abusive use of anorexic agents has grown in the last decade, mainly because of their use in the treatment of obesity. In a survey made by the World Health Organization (WHO)[1,2] this fact has been pointed out. The consumption of anorectics in Brazil is approximately 23.6 tons per year, surpassed only by Chile and Germany.[1,3] About 60% of the world's fenproporex production is used by pharmaceutical laboratories and galenical pharmacies in Brazil. The galenical pharmacies very often commercialize formulas, claimed to be natural, containing only plant components, but omitting the fact that they contain amphetamines and benzodiazepinics substances.

Two methods are described in the literature[4,5] for the quantification of anorectics and benzodiazepinics in pharmaceutical preparations containing plant materials. The first,[4] consists of several experimental steps and quantifies amfepramone hydrochloride (diethylpropion), fenproporex, diazepam, and phenolftalein, using volumetric and spectrometric techniques. The second method[5] describes the determination of amfepramone hydrochloride, fenfluramine, fluoxetin, clobenzorex, diazepam, and flurazepam by thin layer chromatography (TLC) and HPLC. The experimental steps comprise, in addition to extraction and filtration, clean-up steps using cartridges of polyamide. However this procedure does not eliminate all interfering compounds. Mean recoveries for amfepramone hydrochloride and diazepam were 95.8 and 94.0% at fortification levels of 3.35, 9.99, 20.44 mg (amfepramone hydrochloride), and 2.28, 5.26, 9.94 mg (diazepam).

Analysis of preparations containing more than one component, are usually difficult because of the need for sample treatment before the constituents are separated. The objective of the present work was to develop a simple, fast, and efficient method for the determination of amfepramone hydrochloride, fenproporex, and diazepam in pharmaceutical preparations containing herbal components.

EXPERIMENTAL

Chemicals and Materials

Amfeprapramone hydrochloride (99.1%) and diazepam (99.5%) were provided by Medley Indústria Farmacêutica (Brazil), fenproporex (99.3%) was provided by Asta Medica Ltda (Brazil) and used without further purification. Acetonitrile (Merck) and methanol (Merck) HPLC grade, water filtered

through a Milli-Q apparatus (Millipore), and ammonium carbonate (Reagen), analytical grade were used. Stock solutions of standards were made up in methanol using the following concentrations: amfepramone hydrochloride (1000; 2500; 5000, and 15000 $\mu g.mL^{-1}$), fenproporex (1000; 2000 and 6000 $\mu g.mlL^{-1}$), and diazepam (340 and 1000 $\mu g.mL^{-1}$). The working solutions were prepared by dilution of the stock solutions and stored at -17°C.

Apparatus and Chromatography

The HPLC system (Waters) consisted of a Model 501 solvent delivery system, a Model U6K universal injector, a Model 486 UV detector set at 230 nm, and a Model 746 integrator. A LiChrospher 100 RP-18 (Merck) stainless steel column (125 x 4 mm id., 5 μm) and a guard C_{18} (5 μm) guard column (Merck) was used.

The mobile phase containing acetonitrile, methanol, and aqueous 0.1% ammonium carbonate (50:10:40, v/v/v) was filtered through a 0.45 μm Millipore filter and degassed in a Thornton T14 ultrasonic-bath before use. The separation was performed isocratically at a flow rate of 1.0 $mL.min^{-1}$, and a column temperature of 25 ± 1°C.

Analytical Procedure

Five capsules were weighed and homogenized using a mortar and pestle. The correspondent weight of one capsule was transferred into an Erlenmeyer and 15 mL of methanol was added. The flask was then placed in an ultrasonic bath for 20 min. and the extract was filtered through cotton and diluted to 50 mL in a volumetric flask. The solution was then filtered through a 0.45 μm Millipore filter, and 5.0 μL was analyzed by HPLC.

HPLC Analysis

Quantification was performed using a six-point linear calibration curve, plotting concentration vs. peak area. The concentration range was 20 to 2000 $\mu g.mL^{-1}$ for amfepramone hydrochloride, 8 to 800 $\mu g.mL^{-1}$ for fenproporex, and 4 to 200 $\mu g.mL^{-1}$ for diazepam.

Recovery Assays

Control samples containing the herbal components most frequently commercialized in the treatment of obesity, were selected for the recovery assays. The formulation selected is shown below.

Fucus vesiculosus, 58.0 mg

Centella asiatica L., 48.0 mg

Cynara candunculus, 77.0 mg

Passiflora L., 20.0 mg

Rhamnus purshiana DC., 15.0 mg

Saccharomyces cerevisae, 32.0 mg

Excipient (starch), q.s.p., 258.0 mg

Fortified samples were prepared by weighing ten capsules and adding the required standard. The samples were homogenized (using a mortar and pestle) and the correspondent weight of one capsule was submitted to the analytical procedure.

RESULTS AND DISCUSSION

Development of the proposed method was based on the method previously developed in our laboratory[6] for the determination of amfepramone hydrochloride, mazindol, and diazepam in tablets.

The efficiency of the method was evaluated by means of recovery studies with control samples fortified at three different levels: therapeutic dose, one fourth of the therapeutic dose, and three times that of the therapeutic dose.

The recovery and repeatability data are summarized in Table 1. All the active ingredients were extracted from matrices with mean recoveries ranging from 87.1 to 107.8%. Variation coefficients (CV) ranged from 1.2 to 6.2% and show the precision of the method. The detection limits for amfepramone hydrochloride, fenproporex, and diazepam, determined by the described procedure[7] were 0.49; 0.38, and 0.056 mg, respectively. These values are in good agreement with the International Conference on Harmonisat of Technical Requirements for Registration of Pharmaceuticals for Human Use[8] and results reported by Chasin et al.[9] In addition, chromatograms obtained for anorectic determinations were free of interfering peaks at the t_R range of the drugs as exemplified in Figure 1.

A good separation of amfepramone hydrochloride, mazindol, diazepam, and fenproporex was achieved by using the chromatographic conditions described in Section 2.2. The t_R for fenproporex, diazepam, and amfepramone

Table 1

Recovery Efficiency of Active Agents at Level Selected

Drug	Amount Spiked (mg)	Amount Found (mg)	Mean Recovery[a] (%)	(CV)[a] (%)
Amfepramone	25.00[b]	-26.96	107.8	5.1
	6.25	6.29	-100.6	2.6
	75.00	71.7	95.6	1.5
Fenproporex	20.00[b]	17.68	88.4	3.8
	5.00	4.35	87.1	2.5
	30.00	32.19	107.3	1.2
Diazepam	5.00[b]	4.65	93.0	6.2
	1.25	1.22	97.6	1.5
	10.00	9.43	94.3	1.9

[a] n = 8 analyses. [b] These values correspond to the therapeutic doses.

hydrochloride were 2.1, 3.2, and 5.5, respectively. Figure 2 shows a chromatogram of the standard solution analyzed under such conditions.

The HPLC response was found to be linear over the concentration range examined, and the correlation coefficients were 0.9980, 0.9992, and 0.9972, for amfepramone hydrochloride, fenproporex, and diazepam, respectively. The regression analyses of the data gave the slope and interception as:

amfepramone hydrochloride: $Y= 36.4069 X + 8.4544$

fenproporex: $Y= 17.8417 X + 0.4124$

diazepam: $Y= 14.6012 X - 0.4245$

where Y is the peak area and X is concentration.

The proposed method requires only two experimental steps (extraction and filtration), and offers extracts than can be assayed by HPLC in approximately 10 min., in a total analytical time of 1 hour. Therefore, the method has advantages such as time saving, reduction of solvents required for extraction, and unnecessary clean-up steps. The method is efficient, selective, and can be

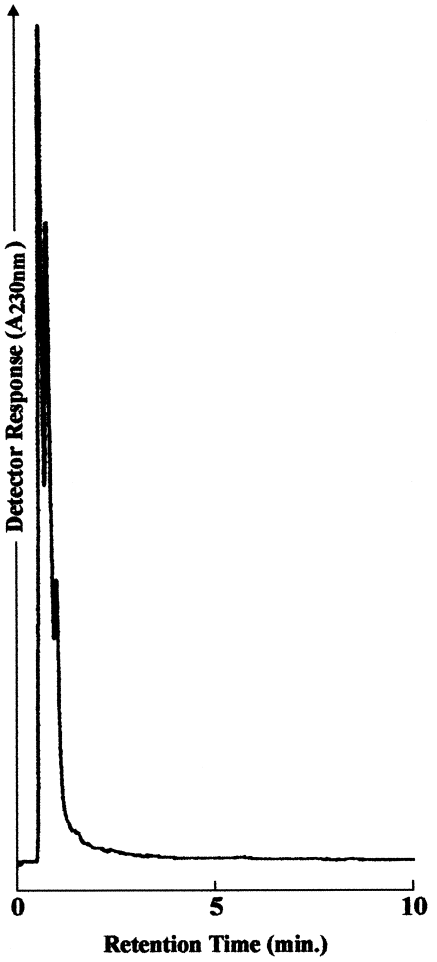

Figure 1. HPLC chromatographic separation of a sample containing only herbal compounds.

employed in studies to determine the presence of anorectics in pharmaceutical preparations containing herbal components.

Twenty samples of pharmaceutical preparations labeled as "natural products" acquired in the region of Araraquara were analyzed. Although the sample

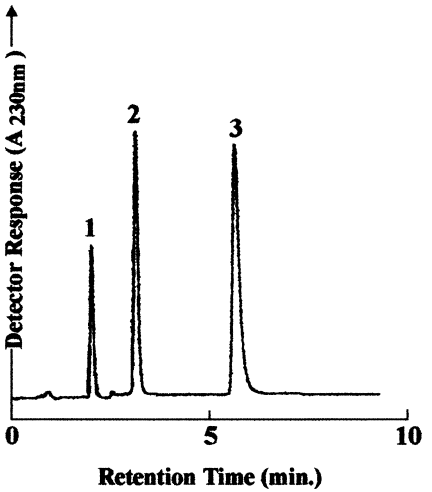

Figure 2. Chromatographic separation of standard solutions: (1) fenproporex; (2) diazepam; (3) amfepramone hydrochloride.

composition declares only herbal components, fenproporex, diazepam, and amfepramone hydrochloride were detected in 35, 20, and 5% of the analyzed samples, respectively.

Phenolftalein, generally used as a laxative, was qualitatively detected in 20% of the samples. Figure 3 shows a chromatogram corresponding to a pharmaceutical preparation containing fenproporex and diazepam.

The most common combination found was diazepam and fenproporex (20%), that is in accordance with Aurichio et al.(39%),[10] in spite of the fact the origin of the samples were significantly different. The samples analyzed qualitatively, by Aurichio[10] were taken from patients that had shown side effects after use. Anorectics were detected in 50% of them. The samples selected for this study were bought in pharmacies in which the identity of the purchases was unknown to the pharmacist. Anorectics and diazepam were detected in 40% of the samples analyzed.

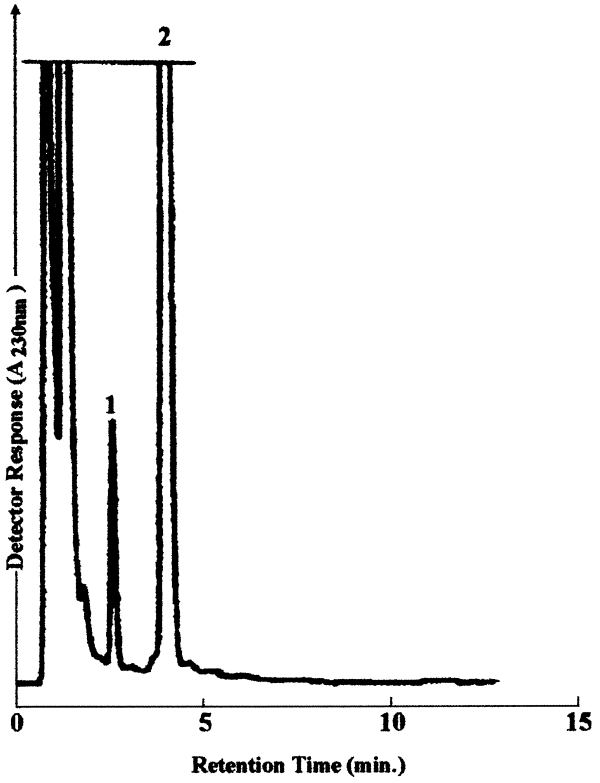

Figure 3. HPLC chromatographic separation of a sample containing herbal compounds (1) fenproporex and (2) diazepam.

In addition to the fraudulent use of components not declared on labels, in severe violation of the law, the use of these "Magic Formulas" can offer serious risks to the health of the patients.

CONCLUSION

Using the chromatographic system employed the separation of amfepramone hydrochloride , fenproporex, and diazepam can be accomplished in approximately 8 min.

The recovery data and variation coefficients of the analytical procedure, developed for pharmaceutical preparations using plant components, assure the

efficiency and reliability of the proposed method. In addition, it presents certain advantages such as simplicity in the handling of samples and time saving. The results obtained analyzing twenty pharmaceutical preparations labeled as "natural products" showed the presence of anorectics and diazepam in 40% of the samples.

The main contribution of the present paper is to propose a simple, fast, and efficient method, for analyzing formulas used for weight loss, that could include the majority of medical prescriptions prepared in galenical pharmacies in the last ten years.

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