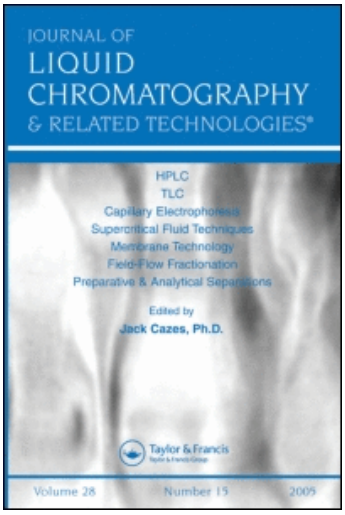

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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF AMFEPRAMONE HYDROCHLORIDE, MAZINDOL, AND DIAZEPAM IN TABLETS

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ABSTRACT

Simple and rapid procedures were developed for the quantification of amfepramone hydrochloride and diazepam and mazindol and diazepam in tablets using high performance liquid chromatography (HPLC) with UV detection. These techniques provided conditions for the separation of each active ingredient from the complex matrices of the dosage forms by dilution or extraction in methanol. Isocratic reversed phase chromatography was performed using acetonitrile, methanol, and aqueous 0,1% ammonium carbonate (70:10:20, v/v/v) as a mobile phase, Radial-Pak C_{18} column (100 x 8 mm id, 4µm), a column temperature of 25±1°C and detection at 255 nm. The calibration curves were linear over a wide concentration range (100-1000 $\mu g.mL^{-1}$ to amfepramone hydrochloride and mazindol and 10-100 $\mu g.mL^{-1}$ to diazepam) with good correlation factors of 0.9978, 0.9956 and 0.9997 for amfepramone hydrochloride, mazindol, and diazepam, respectively.

Mean recoveries obtained from the two kinds of samples ranged from 83.2 to 102.5%, with coefficients of variation ranging from 1.0 to 6.1.

These results demonstrated the efficiency of the proposed methods, as well as advantages such as simplicity and short duration of analysis.

INTRODUCTION

The abusive use of anorexic agents has grown in the last ten years, especially due to their extensive use in the treatment of obesity. Until 1994 the appetite depressors were widely commercialised using several associations, some of which contained an anxiolytic agent: diazepam.

The amphetamine derivatives have been widely investigated using spectrometric[1,2] and chromatographic[3-10] techniques, mainly in biological fluids because of toxicological and forensic interest. There are only three publications in the literature on determination of anoretics in dosage forms.[11-13]

Analyses of preparations containing more than one component are usually difficult because of the previous steps involving sample treatment required for separation of the constituents. Many methods for determination of amfepramone hydrochloride, mazindol, and diazepam in tablets using thin layer chromatography (TLC) have been described in the literature.

The aim of the present study was to develop a simple, fast, and efficient method for determinations of amfepramone hydrochloride and diazepam and mazindol and diazepam in tablet forms. Commercial samples of the pharmaceuticals were selected from those most consumed by the public.

EXPERIMENTAL

Chemicals and Reagents

Amfeprapramone hydrochloride (assigned content 99.1%), mazindol (99.9%), and diazepam (99.5%) were provided by Medley Pharmaceutical Industry (Brazil) and used without further purification. Acetonitrile (Omnisolv), methanol (Omnisolv) and water were of HPLC grade, and ammonium carbonate (Reagen) was of analytical grade.

Chromatography

A Waters HPLC system was used consisting of the following components: two Model 501 pumps, a Model U6K universal injector, a Model 8x10 RCM™ Cartridge Holder, a Model 486 UV detector set at 255 nm, and a Model 746 data system. Chromatographic separation was accomplished using, 100 x 8 mm id Nova-Pak C_{18} Radial-Pak cartridges column with 4µm packing and guard column Nova-Pak C_{18} (4µm).

Chromatographic Conditions

The mobile phase containing acetonitrile, methanol, and aqueous 0.1% ammonium carbonate (70:10:20, 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 $mL.min^{-1}$, a column temperature of 23±1°C.

Standard Solutions

Stock solutions of standards were prepared in methanol in the following concentrations: amfepramone hydrochloride (1000; 2500; 5000, and 15000 $\mu g.mL^{-1}$), mazindol (400; 1000, and 1200 $\mu g.mL^{-1}$), and diazepam (340 and 1000 $\mu g.mL^{-1}$). The working solutions were prepared by dilution of the stock solutions and all the solutions were stored at -17°C until use.

Quantifications were achieved by regression analysis of the peak areas of each compound against concentration and triplicate injections were made.

Calibration curves, were constructed which were linear over the concentration range under investigation, being 100 to 1000 $\mu g.mL^{-1}$ for amfepramone hydrochloride and mazindol, and 10 to 100 $\mu g.mL^{-1}$ for diazepam.

Analytical Procedures

Amfepramone/diazepam tablets

Ten tablets each one containing 75 mg of amfepramone hydrochloride and 10 mg of diazepam were pulverized using a mortar and pestle. An aliquot of this finely ground tablet was weighed and claimed to contain 25 mg of amfepramone hydrochloride and 3.3 mg of diazepam was transferred into an Erlenmeyer, and 15 mL of methanol were added. The flask was then placed in

Table 1

Mobile Phases Tested for Separation of Active Agents

System	Acetonitrile	Methanol	Aqueous 0.1% Ammon. Carb.	Water
A_1	3.5	0.5	6.0	---
A_2	7.0	1.0	2.0	---
A_3	6.0	1.0	3.0	---
A_4	5.0	1.0	4.0	---
A_5	4.0	1.0	5.0	---
B_1	3.5	---	---	6.5
B_2	5.0	---	---	5.0
B_3	6.5	---	---	3.5

an ultrasonic bath for 20 min. and the extract was filtered through cotton and diluted to 50 mL in a volumetric flask. From the samples with amfepramone hydrochloride and diazepam an aliquot of 5.0 mL of extract was diluted to 10.0 mL with methanol. The solution was filtered through a 0.45 µm Millipore filter and 5.0 µl were analyzed by HPLC.

Mazindol/diazepam tablets

The average weight of at least 20 tablets of 1.0 or 1.5 or 2.0 mg of mazindol and 5.0 mg of diazepam were obtained, and previously pulverized, transferred to an Erlenmeyer, and 15 mL of methanol were added. The flask was placed in an ultrasonic bath for 20 min. and the extract filtered through cotton and diluted to 50 mL with methanol in a volumetric flask.

The solution was filtered through a 0.45 µm Millipore filter and 20.0 µL and 5.0 µL were analyzed by HPLC for the quantification of mazindol and diazepam, respectively.

Recovery Analyses of Amfepramone Hydrochloride and Diazepam from Fabricated Placebo Tablets

Placebo samples containing 75.0 mg of amfepramone hydrochloride, 10.0 mg of diazepam, and 9.0 mg of both talcum and magnesium stearate, 90.0 mg of starch, and 257.0 mg of lactose were prepared and analyzed by the HPLC procedure.

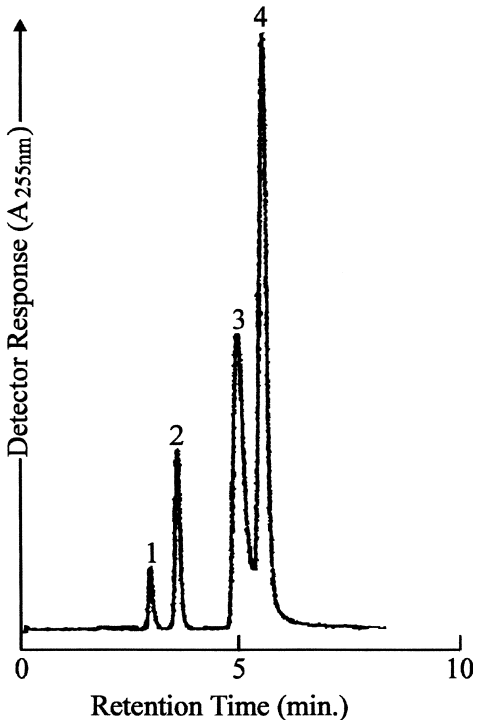

Figure 1. Chromatographic separation of standard solutions: (1) fenproporex, (2) diazepam, (3) mazindol, and (4) amfepramone hydrochloride.

Recovery Analyses of Mazindol and Diazepam from Fabricated Placebo Tablets

Placebo samples containing 1.5 mg of mazindol, 5.0 mg of diazepam, and 6.0 mg of both talcum and magnesium stearate, 60.0 mg of starch, and 221.5 mg of lactose were prepared and analyzed by the HPLC procedure.

RESULTS AND DISCUSSION

Walters and Walters[11] developed a method for the determination of amfepramone hydrochloride and two decomposition compounds in tablets. The extraction was made using methanol in an ultrasonic water bath and the substances were analyzed by HPLC. Two decomposition compounds, 1-phenyl-1,2-propanedione and diethylamine, were identified.

Table 2

Recovery Efficiency of Active Agents from Commerical Samples

	Amfepramone-Hydrochloride		Diazepam	
Sample	Amount Found* (mg ± SD)	Label Claim* (% ± CV)	Amount Found* (mg ± SD)	Label Claim* (% ± CV)
C_1[a]	71.6 ± 4.6	95.5 ± 5.2	10.2 ± 4.8	102.5 ± 5.1
C_2[b]	65.0 ± 3.0	86.7 ± 3.5	9.0 ± 3.0	90.4 ± 3.3
C_3[c]	74.0 ± 4.4	98.7 ± 4.5	----	----

* n = 6 analyses.
[a, b] amounts of amfepramone(75 mg) and diazepam (10 mg).
[c] amounts of amfepramone hydrochloride (75 mg).

Other procedures using UV, IR, GC, and NMR later confirmed the identity of the compounds. Results obtained in the assay using HPLC, related to labeled value, varied from 81.7 to 96.3% and were compared with those obtained using volumetric assays (96.8 - 101.0%) and UV-spectrometry (98.9 - 82.4%).

Based on chromatographic data described in the literature,[11] for amfepramone hydrochloride, various mobile phases and three columns (100 x 8 mm id Nova-Pak C_{18} Radial-Pak cartridges 4µm; 150 x 3.9 mm id Nova-Pak C_{18} stainless steel 4µm; 125 x 4 mm id LiChrospher 100 RP-18 5µm) were tested in order to establish ideal analytical conditions. Initially, experiments were conducted using only standard solutions. As to the mobile phase, two systems using different proportions of acetonitrile, methanol, and ammonium carbonate solution 0.1% (system A) or acetonitrile and water (system B), were tested (Table 1).

Separation of amfepramone hydrochloride, mazindol, diazepam, and fenproporex was achieved by using system A_2, Radial-Pak C_{18} column and detection at 255 nm. The fenproporex was included in the analysis because Brazil imports about 60% of mundial production of this drug[14] and its consumption is high in the country.[15-18] Figure 1 shows a chromatogram of standards obtained under such conditions.

In order to establish experimental parameters for the analytical methodology of anorectics and diazepam in commercial pharmaceutical preparations, two extraction methods[11,12] for pharmaceutical dosage forms were tested.

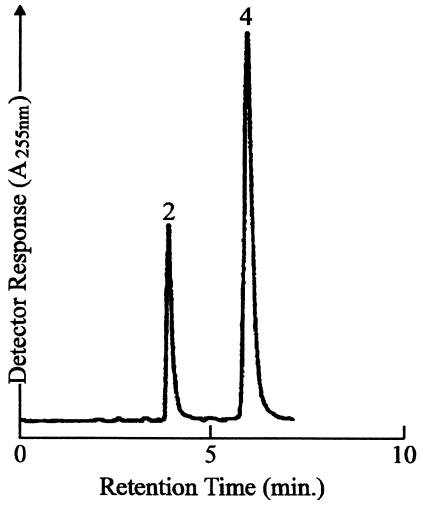

Figure 2. HPLC chromatographic separation of a sample containing (2) diazepam and (4) amfepramone hydrochloride.

The method described by Hoojewijs and Massart[12] tested fenfluramine in Ponderal.® An adaptation of this method proved to be unsatisfactory in this study since data on recovery were not reproducible for amfepramone hydrochloride (68 and 78%) and diazepam (79 and 90%).

The extraction of amfepramone hydrochloride with methanol in an ultrasonic water bath, by means of HPLC, using procaine hydrochloride as an internal standard was described by Walters and Walters.[11] The experimental conditions established for the four active ingredients were based on the principle of this method, and good separation was obtained as shown by the following retention time; 3.5 min. for fenproporex; 4.2 min. for diazepam; 5.1 min. for mazindol, and 6.2 min. for amfepramone hydrochloride.

The correlation coefficients were 0.9978, 0.9956, and 0.9997, for amfepramone hydrochloride, mazindol, and diazepam respectively. The regression analyses of the data gave the slope and intercept as:

amfepramone hydrochloride: $Y= 2.4742 X + 0.1793$

mazindol: $Y= 2.4107 X + 0.2943$

diazepam: $Y= 122.6964 X - 0.4910$

Table 3

Recovery Efficiency of Active Agents from Commercial Samples

	Mazindol		Diazepam	
Sample	Amount Found* (mg ± SD)	Label Claim* (% ± CV)	Amount Found* (mg ± SD)	Label Claim* (% ± CV)
C_4[a]	1.3 ± 5.3	87.2 ± 6.1	4.7 ± 4.9	93.7 ± 5.2
C_5[b]	0.83 ± 0.8	83.2 ± 1.0	----	----
C_6[c]	1.7 ± 1.4	84.2 ± 1.7	----	----

* n = 6 analyses.
[a] amounts of mazindol (1.5 mg) and diazepam (5.0mg).
[b] amounts of mazindol (1.0 mg).
[c] amounts of mazindol (2.0 mg).

where Y and X are the peak area and concentration respectively. In an attempt to detect interferences, simulated samples and placebos were prepared and analyzed. Excipients used in these preparations were those most commonly used in the pharmaceutical industry. The presence of lactose, starch, talcum, and magnesium stearate did not interfere in the results of the analyses.

Simulated samples, containing the same amounts as those used in commercial samples of amfepramone hydrochloride and diazepam, were used to calculate recovery values and presented average values of 90.0 and 96.0% respectively, while the recovery averages for the simulated sample containing mazindol and diazepam were 85.1 and 96.1% respectively. Results referred to the average of three assays and are in good agreement with the values acceptable in the validation of an analytical procedure (80 - 120%).[19-21]

This preliminary study allowed the application of the method developed in six commercial samples designated: C_1, C_2, C_3, C_4, C_5, and C_6. None of the pharmaceutical products contained fenproporex, as it is not presently available on the market.

Table 2 summarizes the results of the study of amfepramone hydrochloride, and diazepam in pharmaceutical preparations and proves the efficiency of the experimental procedure studied. Mean recoveries from six replicates ranged from 86.7 to 102.5%, with coefficients of variation (CV) ranging from 3.3 to 5.1. The results obtained demonstrate the efficiency of the proposed method. The chromatogram and the results of the analyses are shown in Figure 2.

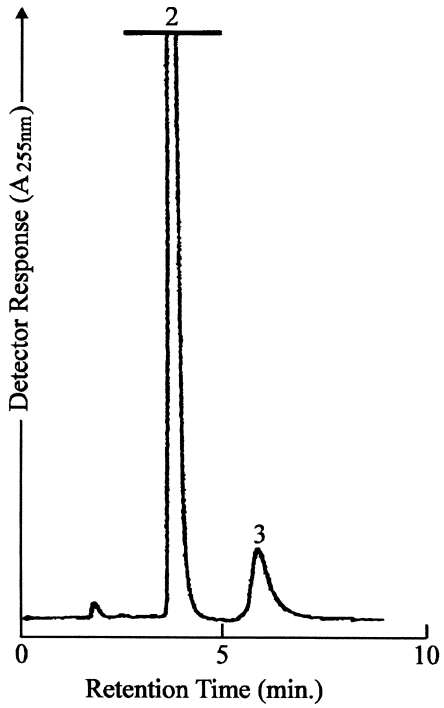

Figure 3. HPLC chromatographic separation of a sample containing (2) diazepam and (3) mazindol (quantification of mazindol).

Table 3 summarizes the results of the mazindol and diazepam studies in pharmaceutical preparations: excellent recovery data (83.2 – 93.7%) and repeatability indicated by coefficients of variation (1.0 – 6.1). The chromatograms and the results of analyses are shown in Figure 3.

CONCLUSION

The HPLC method developed in this study is shown to be simple, precise, and efficient facilitating the determination of amfepramone hydrochloride, mazindol, and diazepam in tablets, eliminating the time-consuming steps involved in previous assay methods.

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