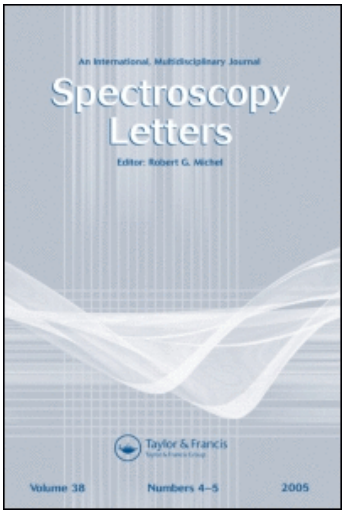

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Quantitative PMR Spectroscopy of Drigs in Pharmaceutical Forms. Determination of Fenfluramine, Diethylpropion, Methyldopa and 2-Acetoxy-4-Trifluoromethylbenzoic Acid

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QUANTITATIVE PMR SPECTROSCOPY OF DRUGS IN PHARMACEUTICAL FORMS. DETERMINATION OF FENFLURAMINE, DIETHYLPROPION, METHYLDOPA AND 2-ACETOXY-4-TRIFLUOROMETHYLBENZOIC ACID.

KEYWORDS: Quantitative ^{1}H NMR spectroscopy; fenfluramine HCl, diethylpropion HCl, methyldopa, 2-acetoxy-4-trifluoromethylbenzoic acid in pharmaceutical dosage forms.

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ABSTRACT

The PMR technique was applied to the quantitative analysis of some drugs, fenfluramine HCl, diethylpropion HCl, methyldopa and 2-acetoxy-4-trifluoromethylbenzoic acid in pharmaceutical forms.

The proposed methods entails a minimum of procedural steps for the extraction of the active ingredients and it is based on the integration of selected proton resonances of the analyte and the internal standard.

INTRODUCTION

From the number of relevant papers in the last few years, there is evidence of an increasing interest in the use of ^{1}H NMR spectroscopy for the quantitation of pharmaceuticals and, in some cases, for the detection (together with chemical identification) of impurities in pharmaceuticals (1-7).

The quantitative analysis of β-adrenergic blocking agents by ^{1}H NMR spectroscopy has been the object of our first paper (8).

The present work describes a quantitative method for the evaluation of some drugs in pharmaceutical formulations by ^{1}H NMR (Table 1). Details of the extraction procedures of the active ingredients from tablets or capsules, the proposed internal standard and the selected region of the spectra for integration are also reported. When possible the determinations were carried out in non-deuterated solvents.

Fenfluramine.HCl and diethylpropion.HCl are anorexigenic drugs, used as adjunct to diet in the management of obesity. Both are commercially available in the form of tablets or capsules. The B. P. 1973 (9) describes a GLC method for the assay of fenfluramine HCl in tablets. Other GLC (10) or spectrophotometric (11) (colorimetric) procedures were used for the determination of the drug in plasma or pharmaceutical dosage forms. GLC (12-13) and a phototurbidimetric method (14) have been also described for the determination of diethylpropion. Proton and ^{13}C NMR (15) spectroscopic methods were reported for the identification of drugs of similar appearance from controlled substances as diethylpropion and others anorexigenic drugs. HPLC (16-17), spectrophotometric (18-23), titrimetric (24-27) and anodic voltametry (28) methods were developed for the determination of methyldopa, an antihypertensive agent, in pharmaceutical formulations.

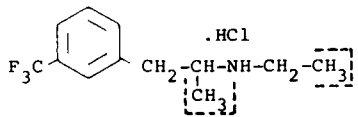
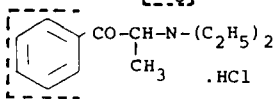
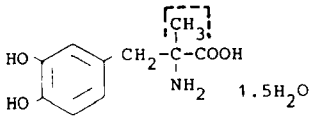
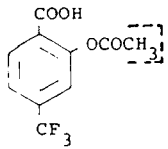
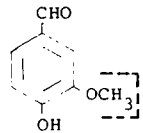
EXPERIMENTAL

Materials. All reference substances and non-deuterated solvents were of analytical grade purity. Deuterated chloroform and deuterium oxide, of 99% and 99.75% of isotope purity respectively, were purchased from Merck.

When chloroform was used for recording the spectra, it was washed with water (5x5 ml for 100 ml of chloroform) to remove the ethanol contained as stabilizer, and then dried over sodium sulphate. In this way, the ^{1}H NMR spectrum of the solvent alone (spectrum amplitude 500, RFP 0.05) did not show the presence of ethanol.

TABLE 1

Proton groups[a] chosen for quantitative analysis of some drugs.

Compound[b]	Structure	E.W.[c]	Internal Standard	E.W.[c]	Solvent for NMR sp.
Fenfluramine.HCl		267.72/6	$C_6H_5NHCOCH_3$	135.16/3	Pyridine
Diethylpropion.HCl		241.75/5	(d)		$CDCl_3$
Methyldopa sesquihydrate		238.24/3	$CH_3COONa.3H_2O$	136.08/3	H_2O
2-acetoxy-4-trifluoro methylbenzoic acid		248.16/3		152.14/3	$CHCl_3$

(a) Dotted lines indicate protons selected for the analysis
(b) Indicated in the form (base or salt) present in the pharmaceuticals
(c) Equivalent weight = M.W./number of protons under integration
(d) Determined by means of a calibration curve (external standard)

H NMR spectra: The spectra were recorded in the established solvent (Table 1), in 5 mm tubes, with a 60 MHz Varian T-60 continous wave spectrometer. All precautions to assure accuracy of integration were taken, including high signal-to noise ratio, correct phasing, adjustment of magnetic field homogeneity and spin rate, so that spinning side bands were minimized in the operating region and might be neglected. The analytical signals were integrated four times and the values averaged. This operation was repeated at different integral amplitudes.

When pyridine, chloroform or deuterochloroform were used as solvents the δ scale was referenced to TMS, in D_2O to DSS, adjusted to 0 ppm. The average integral values of at least 4 replicates were used to calculate the quantity of drug under study using the following equation:

$$\frac{\text{mg X}}{\text{tablet}} = \frac{I_x}{I_{std}} \cdot \frac{EW_x}{EW_{std}} \cdot \frac{\text{mg std}}{\text{mg powder}} \cdot \text{average tablet weight}$$

where I_x = average integral value of substance under study; I_{std} = average integral value for internal standard; EW_x and EW_{std} = equivalent weight of component and internal standard respectively.

Extraction procedures:

Fenfluramine.HCl. Ten tablets were weighed and finely powdered. A portion of powder, equivalent to 60 mg of fenfluramine.HCl, was accurately weighed into a 50 ml Erlenmeyer flask, 30 ml methanol was added and the mixture shaken for 30 min. After that, 5 ml of 0.05N methanolic solution of $AgNO_3$ (equivalent amount plus 1-2% excess for neutralization of the hydrochloric salt) was added, the mixture shaken for 2 min and then left standing for 10 min. After addition of acetanilide (ca 60 mg, accurately weighed) as internal standard, the suspension was filtered quantitatively under low pressure, through a pad of Celite, and the solution was taken to

dryness on a rotavapor. The residue was dried under vacuum (30 °C, 2 h) and then dissolved in 1 ml of pyridine. About 0.5 ml of this solution was transferred into a 5 mm NMR tube and the spectrum was measured. Four or more independent experiments were carried out for each pharmaceutical formulation. In a similar way, at least once, or in parallel if necessary, a sample was prepared without addition of internal standard to verify the baseline of the spectrum in the region of analytical interest for the internal standard.

Diethylpropion.HCl (amfepramone.HCl). The amount of powdered tablets equivalent to 50 mg of amfepramone.HCl (two tablets) was suspended in 20 ml of chloroform. After addition of few drops of concentrated ammonia (alkaline pH) the mixture was occasionally shaken for 30 min, dried over sodium sulphate and filtered through a cotton pledget. The filtrate was concentrated to dryness under vacuum. The residue dissolved in 0.5 ml of $CDCl_3$ was used for the spectrum.

The concentration of the drug was established by reference to a calibration curve constructed by plotting integral values against known amounts of diethylpropion (at least 3 concentrations) selected in the range of the expected concentration of the drug. The registration of the related spectra should be carried out consecutively with the instrument set at the same parameters.

A pure sample of diethylpropion was obtained by extraction with chloroform of 10-20 powdered tablets suspended in aqueous ammonia. The organic extract was dried over sodium sulphate, filtered through a cotton pledget and concentrated under vacuum to leave an oil which was distilled in a ball tube oven at 105 - 110 °C, 0.01 mm Hg (TLC, $CHCl_3$-CH_3OH-NH_4OH (95:5:0.5), RF 0.74, visualization with UV (long wavelenght) and Dragendorff's reagent).

Methyldopa. Ten tablets were weighed and finely powdered. A portion of powder equivalent to 250 mg of anhydrous methyldopa was

accurately weighed into a 100 ml Erlenmeyer flask, 50 ml of water was added and the mixture shaken for 30 min. The suspension was filtered under low vacuum through a pad of Celite and the solution, after the addition of few drops of 6N HCl, was concentrated on a rotavapor to dryness. To the residue dissolved in 5 ml of water, was added sodium acetate trihydrate (ca 140 mg, accurately weighed) as internal standard and 0.5 ml of this solution used for the spectrum.

The methyldopa content in tablets was also determined by using a calibration curve obtained by plotting integral heights against three concentrations of methyldopa, one corresponding to 50 mg (calculated on anhydrous basis) and the other two to ±35% of the theoretical value in D_2O-DCl (9:1).

2-Acetoxy-4-trifluoromethylbenzoic acid (Trifusal). To about 60 mg of powder, from the mixed content of ten capsules (with no other ingredient or excipient) ca 37 mg of vanillin, as internal standard, was added. The mixture was dissolved in 1 ml of $CDCl_3$ and 0.5 ml of this solution used for the spectrum.

UV spectroscopy. Absorbance values were measured in 1 cm silica quartz cells, using a Perkin Elmer 402 UV-Visible recording spectrophotometer.

The amount of powder corresponding to 60 mg of fenfluramine HCl, was transferred to 100 ml volumetric flask. After addition of water (50 ml), the flask was shaken for 30 min and then taken to volume. The solution was filtered and diluted to obtain solutions whose absorbance was measured at 264 nm. Concentrations were read through a calibration curve (abs. vs. conc.) obtained from water solutions of pure fenfluramine.HCl, from 5 to 30 mg % (w/v): $y = 0.02x + 0.02$; $n = 5$; $r = 0.999$.

Diethylpropion.HCl was extracted as base from tablets or capsules. An amount of powder corresponding to about 50 mg of drug was suspended in water, sonicated for 15 min and filtered quanti-

tatively. The filtrate was alkalinized with ammonia and extracted with ether. The organic phase was concentrated to dryness on a rotavapor and the residue was dissolved in methanol and diluted to obtain solutions containing 1.0-1.5 mg% (w/v). The concentration was read through a calibration curve obtained by plotting the absorbance at 242 nm against concentrations (0.5 to 2 mg% w/v) of pure diethylpropion dissolved in methanol: y = 0.056x; n = 4; r = 0.999.

The amount of powdered tablets, equivalent to 250 mg of anydrous methyldopa (282 mg sesquihydrate), was transferred to 500 ml volumetric flask. After the addition of water (350 ml), the flask was shaken for some min, and then taken to volume. After standing for 30 minutes, the clear solution was diluted to obtain concentrations of 2.5 and 5 mg % (w/v) whose absorbance was measured (λ_{max} 282 nm). Solutions of pure methyldopa in water, containing from 0.5 to 5 mg % (w/v), were used to construct a calibration curve: y = 0.11x + 0.01; n = 5; r = 0.999.

Potentiometric titration. An automatic potentiometric titrator (Metrohm, Titroprocessor E 636) with a combined glass electrode was used to perform potentiometric determinations. About 50 mg (accurately weighed) of 2-acetoxy-4-trifluoromethylbenzoic acid sample was dissolved in 20 ml of absolute ethanol, 20 ml of water were added and the solution was titrated potentiometrically with 0.02 N sodium hydroxide.

RESULTS AND DISCUSSION

In Table 1 are shown the protons of the drug and internal standard selected for the quantitative analysis.

In Table 2 the results obtained by PMR spectroscopy are compared with the results obtained with alternative methods carried out in our laboratory.

The extraction procedures played an important role in the recovery of the drug. Various solvents were tested, those reported

TABLE 2

Determination of some drugs in commercial tablets or capsules by ^{1}H NMR.

Drug	mg/tablet declared	Internal Standard	mg/tablet found (S.D.)[a]	%found (S.D.)	mg/tablet found (S.D.) (UV method)[a]
Fenfluramine.HCl	20	Acetanilide	19.95 ± 1.00	99.75 ± 5.01	19.15 ± 0.78[b]
	60	"	64.32 ± 0.37	107.20 ± 0.62	56.85 ± 1.15
	20	"	20.15 ± 1.19	100.75 ± 5.96	20.20 ± 0.49
	60	"	61.50 ± 2.63	102.50 ± 4.38	60.96 ± 2.16
Diethylpropion.HCl	25	External standard	24.05 ± 1.61	96.20 ± 6.44	24.55 ± 1.15[d]
Methyldopa 1½ H_2O	250	Sodium acetate.3H_2O	245.90 ± 6.05	98.44 ± 2.42	253.76 ± 6.65[c]
	"	External standard	238.00 ± 8.00	95.20 ± 3.36	
2-acetoxy-4-trifluoro methylbenzoic acid	300	Vanillin	298.35 ± 6.93	99.45 ± 2.31	304.08 ± 1.28[e]

a. From four indipendent experiments
b. At 264 nm
c. At 282 nm
d. At 242 nm
e. Potentiometric tritration with 0.02 N NaOH

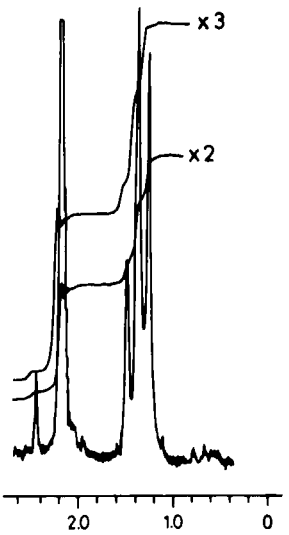


Figure 1: NMR signals used for quantitation of fenfluramine.HCl in the presence of acetanilide (δ 2.20) as reference compound, in pyridine.

for each drug proved to be the most preferable because of smaller interference with excipients.

Fenfluramine.HCl was extracted from tablets or capsules, as a base, with methanol and methanolic silver nitrate, a procedure successfully used for the extraction of several β-adrenergic blocking agents from tablets (8). The analytical region of the spectrum in pyridine is shown in Figure 1.

Diethylpropion.HCl was extracted from tablets with chloroform, as free base. The spectrum in $CDCl_3$ of the final residue showed evidence of excipient interference (magnesium stearate) at δ 1.25 the region where resonate the methyl protons of the drug. For this reason, in this case, the aromatic protons of the drug ranging from δ 7.30 to 8.30 were selected for integration (Figure

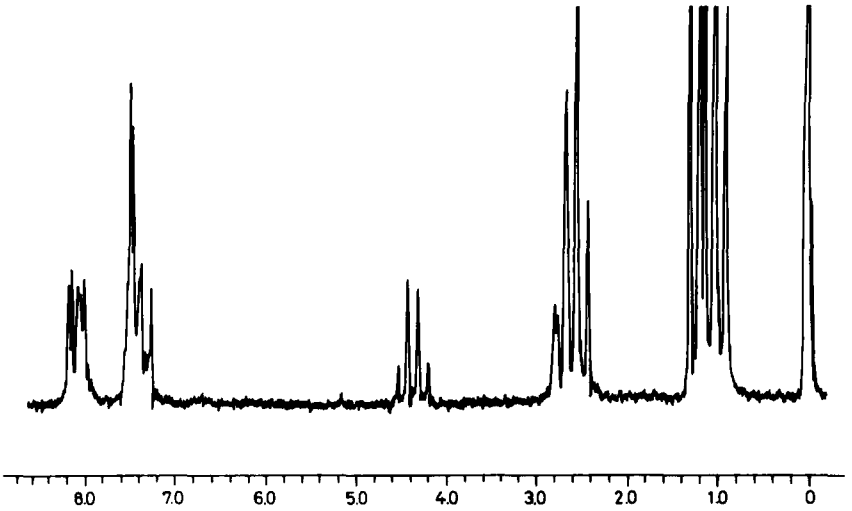


Figure 2: NMR signals used for quantitation of diethylpropion.HCl in deuterochloroform.

2) and quantitation was performed by means of a calibration curve by plotting peak height of the aromatic protons against concentration. The small peak of chloroform can be neglected since the volume of deuterochloroform used for running the spectra was always the same (0.5 ml).

Methyldopa was extracted from tablets with water as hydrochloride and no interference from excipients was observed in the region where the methylprotons of the internal standard, sodium acetate, resonate (Figure 3).

2-Acetoxy-4-trifluoromethylbenzoic acid (trifusal) was present in capsules without excipients. The NMR spectrum of this compound in $CDCl_3$ gave evidence of its molecular structure, and by adding vanillin as internal standard a quantitative analysis was performed (Figure 4).

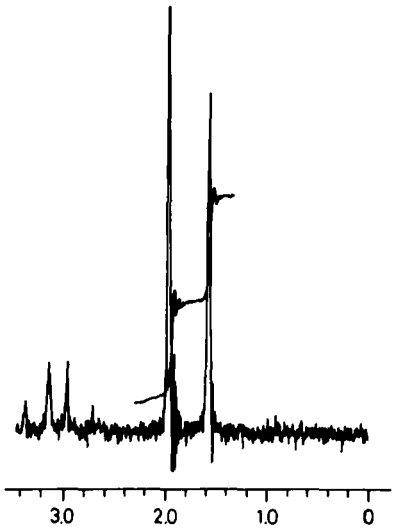


Figure 3: NMR signals used for quantitation of methyldopa in the presence of sodium acetate trihydrate (δ 1.92), as reference compound, in water.

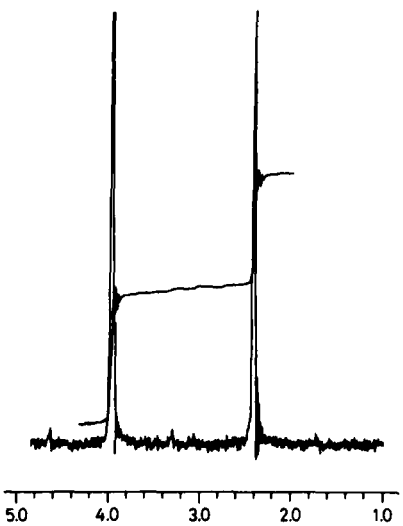


Figure 4: NMR signals used for quantitation of 2-acetoxy-4-trifluoromethylbenzoic acid in the presence of vanillin (δ 4.0), as reference compound, in chloroform.

The proposed method proved to be satisfactory with respect to both accuracy and precision and in agreement with data obtained using different methods (Table 2). In addition, the method is simple and selective since the spectrum gave also evidence of the structure of the drug under examination.

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