The tendency of I to exhibit fluorescence in solution is potentially useful for analysis by HPLC. However, this fluorescence is readily quenched in aqueous solutions, thereby hindering its use in many reversed-phase liquid chromatographic systems. Normal-phase liquid chromatography does not appear promising due to the strong affinity of I for silica gel columns, resulting in asymmetric peak shapes and excessive capacity factors. Thus, paired-ion reversed-phase chromatography in conjunction with detection by UV absorbance is used for the analysis of I by HPLC. The parameters chosen provided good peak shape, a moderate retention time, and analytical sensitivity comparable to that obtained by fluorescence detection.

The selection of one procedure in preference to the other for the analysis of plasma samples from clinical studies is a matter of available equipment and efficiency. TLC is used preferentially to HPLC in this laboratory for the rapid analysis of limited numbers of samples (typically 1–20). Larger numbers of samples may be analyzed more efficiently by HPLC using an auto-injector to provide for unattended operation. The chromatographic equipment is interfaced with microcomputers to facilitate quantification, thereby achieving high levels of precision and accuracy.

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NMR Determination of Isosorbide Dinitrate and β-Adrenergic Blocking Agents in Tablets

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Abstract □ An NMR spectroscopic method for the determination of isosorbide dinitrate, alone or together with alprenolol or propranolol, is described. Spectra are determined in dimethyl sulfoxide-d_6 containing maleic acid or 1,4-dinitrobenzene as internal standards. Both synthetic mixtures and commercial formulations were assayed, and the results were compared using compendial procedures.

Keyphrases □ Isosorbide dinitrate—NMR determination, β-adrenergic blocking agents in tablets □ β-Adrenergic blocking agents—NMR determination of isosorbide dinitrate, tablets □ NMR spectroscopy—determination of isosorbide dinitrate and β-adrenergic blocking agents in tablets

Isosorbide dinitrate (I) is a member of the group of vasodilator drugs, having a nitrite or nitrate function, that are used particularly in the treatment of angina pectoris and ischemia of skeletal muscle (1). It is used alone or together with β-adrenergic blocking agents such as propranolol (II) (2) or alprenolol (III) (3).

Several methods for the analysis of I, II, and III have been described. The official compendia describe a polarographic titration (4) or a colorimetric reaction with *p*-phenolsulfonic acid (5) for isosorbide dinitrate and a spectrophotometric assay for alprenolol (6) and propranolol (7). However, their application for the quantitative determination of I in tablets is laborious and time consuming. The present report describes a new quantitative method for the determination of I alone or together with II or III by ^{1}H-NMR spectroscopy. Furthermore, it allows the simultaneous analysis of II or III.

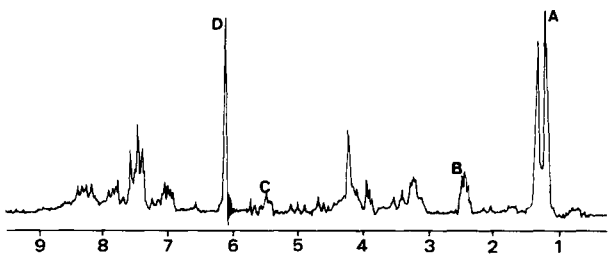

Figure 1—*NMR spectrum of a typical I and II mixture analysis in dimethyl sulfoxide-*d_6. *Key: (A) methyl protons of II; (B) solvent impurity; (C) protons on* C_2 *and* C_5 *of I; (D) single signal of IV.*

EXPERIMENTAL[1]

Materials—Standard isosorbide dinitrate (I), propranolol hydrochloride (II), and alprenolol hydrochloride (III) were purified by recrystallization from ethanol–water, *n*-propanol, and ethyl acetate, respectively.

Maleic acid (IV) was used as internal standard after recrystallization from water, mp 130–131°; 1,4-dinitrobenzene (V) (8), also used as internal standard, was purified by sublimation, mp 173–174°. Dimethyl sulfoxide-d_6 (VI) was used as the solvent and tetramethylsilane was the internal standard.

Samples—Tablets from two batches of each brand locally obtained were used.

Isosorbide Dinitrate Tablets—Twenty tablets were weighed and a sample equivalent to 50 mg of I was dissolved in 10 ml of 2% sodium bicarbonate solution and extracted from methylene chloride (3 × 7 ml). The solution was evaporated in a Craig tube, and 75 mg of IV was added. The mixture was dissolved in 1.0 ml of VI, and ~0.4 ml of the solution was transferred to an NMR tube where the spectrum was obtained (Table I).

Isosorbide Dinitrate and Propranolol Hydrochloride Tablets—The same procedure as just described was used with a sample equivalent

[1] A Perkin-Elmer R12 NMR spectrometer, 60 MHz was used. All spectra were scanned at a probe temperature of 35°.

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Table I—Analysis of Isosorbide Dinitrate (I) Tablets

Sample	Declared dosage, mg	NMR		USP XX[a]		FA VI[b]	
		mg	Percent	mg	Percent	mg	Percent
A	20.0	19.6	98.0	20.1	100.5	20.3	101.4
B	20.0	19.3	96.4	19.6	98.0	19.9	99.5
C	20.0	20.0	100.0	20.4	102.0	20.6	103.2
D	20.0	19.7	98.6	20.3	101.5	20.4	102.1

[a] See Ref. 4. [b] Formacopea Naconial Argentina (see Ref. 5).

Table II—Analysis of Tablets of Isosorbide Dinitrate (I) and Propranolol Hydrochloride (II)

Tablets	Propranolol Hydrochloride					Isosorbide Dinitrate				
	Declared dosage, mg	NMR		BP[a]		Declared dosage, mg	NMR		USP XX[b]	
		mg	Percent	mg	Percent		mg	Percent	mg	Percent
A	40.0	39.9	99.7	40.7	101.8	10.0	10.5	105.3	9.8	97.8
B	40.0	39.7	99.2	39.9	97.5	10.0	10.2	102.2	10.1	100.9
C	40.0	41.2	102.9	41.2	103.0	10.0	10.2	101.9	9.8	98.5
D	40.0	40.0	100.0	39.4	98.4	10.0	9.9	98.7	9.9	99.4

[a] British Pharmacopoeia (see Ref. 7). [b] See Ref. 4.

Table III—Analysis of Tablets of Isosorbide Dinitrate (I) and Alprenolol Hydrochloride (III)

Tablets	Alprenolol Hydrochloride					Isosorbide Dinitrate				
	Declared dosage, mg	NMR		BP[a]		Declared dosage, mg	NMR		USP XX[b]	
		mg	Percent	mg	Percent		mg	Percent	mg	Percent
A	50.0	51.6	103.2	51.1	102.2	10.0	10.5	104.9	10.2	102.5
B	50.0	50.7	101.4	49.9	99.8	10.0	10.3	102.7	10.1	101.1
C	50.0	51.3	102.6	51.4	102.9	10.0	10.3	102.6	9.8	99.7
D	50.0	46.4	92.8	47.6	95.2	10.0	10.3	102.8	10.2	102.0

[a] British Pharmacopoeia (see Ref. 6). [b] See Ref. 7.

Table IV—Analysis of Standard Mixtures of Isosorbide Dinitrate (I) and Propranolol Hydrochloride

Standard Mixture	Internal Standard, mg	Propranolol Hydrochloride			Isosorbide Dinitrate		
		Added, mg	Found, mg	Recovery, %	Added, mg	Found, mg	Recovery, %
1	75.5	188.7	187.0	99.1	40.3	39.2	97.2
2	63.2	204.3	206.8	101.2	46.9	47.8	101.8
3	70.7	233.2	227.3	97.5	50.0	47.2	94.3
4	73.2	204.8	205.0	99.9	47.0	47.2	100.5
5	77.0	209.4	210.0	100.3	55.5	53.1	95.6
6	75.0	238.5	231.1	96.9	41.7	39.7	95.2
7	78.0	201.7	198.2	98.3	49.8	50.2	100.7
8	74.5	202.2	206.0	101.9	51.5	50.5	98.0
				Mean = 99.4			Mean = 97.9
				% *SD* = ±1.80			%*SD* = ±2.90

to 50 mg of I and 200 mg of II using 75 mg of IV as the internal standard (Table II).

Isosorbide Dinitrate and Alprenolol Hydrochloride Tablets—The same procedure as described for isosorbide dinitrate was used, with a sample equivalent to 20 mg of I and 100 mg of III using 60 mg of V as the internal standard (Table III).

Calculations—The amount of I was calculated as follows:

$$\frac{\text{I, mg}}{\text{tablet}} = \frac{EW_I}{EW_{ST}} \times \frac{A_I}{A_{ST}} \times \frac{ST, \text{mg}}{W_S} \times ATW$$

Where EW_I is the molecular weight of I divided by the number of protons in the signal chosen, EW_{ST} is the molecular weight of the internal standard divided by the number of protons in its signal, A_I is the integral value of the signal representing I, A_{ST} is the integral value of the signal representing the internal standard, W_S is the weight of the sample in milligrams, and ATW is the average tablet weight.

The amount of II and III was calculated by a similar procedure.

RESULTS AND DISCUSSION

All the drugs and internal standards were soluble in dimethyl sulfoxide. Its impurity appeared at 2.5 ppm and it did not interfere with the signals chosen. The stability of the drugs in this solvent during testing was ensured by running the spectra twice.

Maleic acid was selected as the internal standard because it provides a signal at 6.2 ppm, which is near the signal chosen for the analysis; therefore, the error due to drift is small. Furthermore, it has an equivalent of 58, larger than the equivalent of most internal standards. For the analysis of III, V was used due to the overlapping of the signals of III and IV.

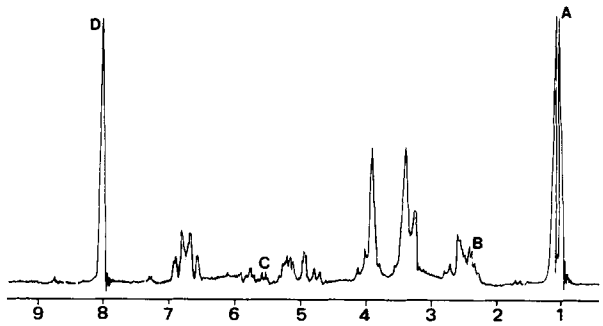

Figure 2—*NMR spectrum of a typical I and III mixture analysis in dimethyl sulfoxide-*d_6. *Key: (A) methyl protons of III; (B) solvent impurity; (C) protons on* C_2 *and* C_5 *of I; (D) single signal of V.*

Table V—Analysis of Standard Mixtures of Isosorbide Dinitrate and Alprenolol Hydrochloride

Standard Mixture	Internal Standard, mg	Alprenolol Hydrochloride			Isosorbide Dinitrate		
		Added, mg	Found, mg	Recovery, %	Added, mg	Found, mg	Recovery, %
1	101.7	253.0	252.5	99.7	53.2	53.5	100.6
2	102.7	250.7	261.0	104.1	53.5	53.5	100.0
3	114.0	258.2	252.2	101.5	52.5	54.2	103.3
4	97.7	260.5	256.5	98.5	57.5	56.5	98.3
5	94.5	251.0	256.5	102.2	57.0	56.0	98.2
6	94.8	252.0	258.0	102.4	57.0	58.5	102.6
7	91.2	249.5	254.0	101.8	52.5	49.0	93.3
8	91.5	258.5	266.0	102.9	62.0	63.0	101.6
				Mean = 101.6			Mean = 99.7
				%SD = ±1.78			%SD = ±3.18

The signal chosen for the analysis of I was the multiplet at 5.5 ppm due to the protons on C_2 and C_5. The presence of this signal at low fields is due to the electronegativity of the nitrate ester group as in 1,4:3,6-dianhydro-D-glucital. In this compound both diacetoxy and dimesyloxy derivatives have their C_2 and C_5 protons shifted to lower fields (9).

The doublet at 1.25 ppm due to both methyl groups was chosen for II. The signal given by the same group in III, a doublet at 1.30 ppm, was selected for III. The singlets at 6.2 ppm for IV and 8 ppm for V were used for the internal standards.

The results of the analysis of a group of known standard mixtures of I and II and I and III are summarized in Tables IV and V (Figs. 1 and 2). The method is accurate and precise, with an $SD \pm 3.06$ for I. The standard deviation is ±1.80 and ±1.78 for II and III, respectively, for the standard mixtures.

This procedure was also applied to commercial lots of tablets from three companies containing I and its mixtures with II and III.

The results obtained are in agreement with those obtained using the official USP procedure for I (4) and the BP procedures for II (6) and III (7).

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Ion-Specific Electrode Study of Copper Binding to Serum Albumins

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Abstract □ The binding of copper to bovine, human, rabbit, rat, and porcine albumin has been studied using a cupric ion-specific electrode. The results were analyzed in terms of Scatchard expression assuming two classes of independent binding sites. The high-affinity constants for copper binding to the albumin show the same trend as the first association constants for nickel binding, namely, rabbit > human > rat > pig. Despite the similarity in the primary amino acid sequence for human and bovine serum albumin, the former has only one high-affinity site for copper, while the latter has more than three sites.

Keyphrases □ Binding—copper to serum albumin, ion-specific electrode study, bovine, human, rabbit, rat, porcine □ Serum albumin—bovine, human, rabbit, rat, porcine, ion-specific electrode study, copper binding □ Ion-specific electrode—copper binding to serum albumin, bovine, rabbit, human, rat, porcine

Serum copper levels play a dual role in several pathological conditions, both in humans and experimental animals (1, 2). Anti-inflammatory properties are associated with several salts and chelates of copper (3–9). On the other hand, increased copper levels have been observed in several diseases such as tuberculosis and pneumonia (10), rheumatoid arthritis (11, 12), and ankylosing spondilitis (13). In serum the weakly bound form of copper is associated with the transport protein, albumin. Sequence analysis studies have shown that the first three amino acids at the *N*-terminal constitute a high-affinity copper binding site in bovine (14–16), human, and rat albumin (17). Nickel, which has been implicated as a carcinogen in humans and experimental animals (18), also binds to this *N*-terminal site.

There have been several previous attempts to measure the binding of copper to serum albumins from different animal species. In an early study (19) the thermodynamics of copper binding to bovine serum albumin using absorption spectroscopy were examined. However, the insensitivity of the spectroscopic method necessitated the use of high protein concentrations, which precluded the determination of the high-affinity binding constants. A later study (20) estimated the number of copper binding sites on bovine and human albumin but the relative affinity constants were not determined. A more recent study (21)

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