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A Sensitive Spectrophotometric Assay for Tinidazole and Metronidazole Using a Pd-C and Formic Acid Reduction System

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A simple, sensitive and rapid spectrophotometric method for the determination of tinidazole (TZ) and metronidazole (MZ) in pure as well as in dosage form is described. The method is based on the reduction of the nitro group of drugs using a novel and versatile reduction system comprising 10% Pd-C and formic acid. The resulting amine was then subjected to a condensation reaction with sodium 1,2-naphthaquinone-4-sulfonate (NQS) to form red Schiff base with an absorption maximum at 510 nm. Beer's law was obeyed in the concentration ranges 2.0 to 45.0 μg mL^{-1} and 1.5 to 37.0 μg mL^{-1} with a limit of detection (LOD) of 0.44 μg mL^{-1} and 0.36μg mL^{-1} for TZ and MZ, respectively. Other statistical analyses such as Student's t test and F test values are included. The sensitivity of the method surpasses that of the reported spectrophotometric methods. The method was successfully applied for the assay of different tablets, suspensions and injections of TZ and MZ.

Key Words: Spectrophotometry, Tinidazole, Metronidazole, Pharmaceutical formulations.

Introduction

5-Nitroimidazoles such as tinidazole and metronidazole are extensively used as antiamoebic, antiprotozoal and antibacterial drugs. The discovery of the antibacterial and antitrichomonal properties of the antibiotic azomycin led to the investigation of nitroimidazoles as antiparasitic agents.[1,2] The discovery of the antitrichomonal properties of metronidazole[3,4]revolutionised the treatment of disease. Although the amoebicidal properties of metronidazole were studied,[3] it was not clinically tested until some years later. In laboratory tests, metronidazole is effective against intestinal amoebiasis in rats and hepatic amoebiasis in hamsters and is also active against *E. histolytica* in vitro.[3,4]The initial clinical tests of metronidazole indicated that it was capable of curing invasive amoebic dysentery and amoebic liver abscess.[5]Subsequent clinical tests have established metronidazole as the drug of choice in the treatment of all forms of amoebiasis in humans.[6,7]

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Variation of the structure of metronidazole, principally to improve trichomonacidal activity and metabolic stability, led to the discovery of tinidazole. Tinidazole is active against *E. histolytica* in vitro,cecal amoebiasis in rats, and hepatic amoebiasis in hamsters.[8]Clinical tests have established the value of tinidazole in the treatment of intestinal and hepatic amoebiasis in humans.[9] Tinidazole has about the same or slightly greater efficacy than metronidazole in the treatment of trichomoniasis and in giardiasis, it has been found to be effective against strains resistant to metronidazole.[10]

Tinidazole and metronidazole are officially determined by titrimetry, potentiometry and HPLC methods. Indian Pharmacopoeia[11](IP) describes the non-aqueous titration method using perchloric acid as titrant and malachite green as indicator for the assay of tinidazole and metronidazole. British Pharmacopoeia[12](BP) describes potentiometric and non-aqueous titration methods using perchloric acid as titrant. United States Pharmacopoeia[13](USP) describes HPLC and non-aqueous titration methods for the assay of metronidazole only. Most of the spectrophotometric methods found in the literature for the determination of tinidazole and metronidazole in the visible region involve initial reduction by treatment with Zn and HCl[14−20] followed by the diazotisation and coupling of the resulting amine. All these methods are less sensitive[14−16,21], involve tedious procedures such as heating and extraction,[19,22,23] utilise costly reagents and involve an additional diazotisation step[24]. The present method is an attempt to overcome the shortcomings of the existing procedures and we succeeded in developing a simple, rapid and accurate spectrophotometric procedure for the assay of tinidazole and metronidazole.

Experimental

Instrumentation and reagents

A JASCO model UVIDEC-610 UV-VIS spectrophotometer (Japan Spectroscopic Co. Ltd., Hachioji, Tokyo, Japan) with 1.0 cm matched quartz cells was used for electronic spectral measurements.

Tinidazole and metronidazole were obtained as gift samples from Sarabhai Pharmaceuticals, Baroda, India. Sodium hydroxide was purchased from BDH and sodium 1,2-naphthquinone-4-sulfonate was purchased from Sigma, 10% Pd-C and formic acid used were purchased from E-Merck, Chennai, India. In addition 0.5 M sodium hydroxide and a freshly prepared 0.05% aqueous solution of sodium 1,2-naphthaquinone-4-sulfonate were used. 90% formic acid was used for the reduction of the drugs. Distilled water was used for the preparation of all aqueous solutions.

Reduction of the nitro group and preparation of standard drug solutions

First 25 mg of each drug was accurately weighed and placed in different 50 mL round bottomed flasks. Then we added 5 mL of methanol and 0.25 g of 10% Pd-C. To the stirring suspension of this mixture was added 5 mL of 90% formic acid and the reaction (exothermic) was carried out at room temperature with constant stirring for 30 min. After the completion of the hydrogenation, the mixture was filtered and washed with methanol and diluted to 100 mL with water.

General procedure

Preparation of calibration curves: Different volumes of the drug solutions (in the range 0.2 to 4.5 mL for TZ and 0.15 to 3.7 mL for MZ) prepared above were accurately measured into different 25 mL volumetric flasks. After the addition of 3.5 mL of 0.05% of NQS, 5 mL of 0.5 M sodium hydroxide was added and diluted up to the mark with water. Absorbance of each solution was then measured after 15 min at 510 nm. A calibration graph was constructed by plotting the absorbance against the concentration of the drug.

Assay procedure: An amount of bulk sample or tablet powder or a volume of injection or suspension exactly equivalent to 25 mg of the drug was extracted with chloroform (3 x 5 mL portions). Combined extracts were then evaporated on a steam bath and the residue was treated as for the preparation of the standard drug solution. Then the procedure reported under 'preparation of calibration curves' was followed for the assay of the drug content. The drug concentration was read from the appropriate calibration graph prepared under identical conditions.

Results and Discussion

Reduction of the nitro group

Progress of the reaction of the reduction was continuously monitored by TLC using a $CHCl_3$, methanol and acetic acid mixture in the ratio 93:5:2 as eluent, and Rf values were calculated. Rf values of tinidazole and metronidazole were 0.85 and 0.9, respectively, and after reduction the Rf values were 0.63 and 0.67, respectively.

Formic acid in the presence of 10% Pd-C is a rapid, versatile and selective reducing system for a wide variety of nitro compounds.[25] This system is readily available and easy to operate, and produces products in good yield with high purity. The obvious advantages of the method over the other reduction procedures are selective reduction of the nitro compounds in the presence of other reducible groups, rapid, high yield, avoidance of strong acids, and the fact that the catalyst can be recovered and reused after washing with water and ethanol without appreciable loss of activity.

Determination of absorption maximum

Reduced drugs when treated with NQS form a red product (Schiff base) in alkaline medium. To determine absorption maximum, 10 μg mL^{-1} of each drug solution was reacted with NQS. After 15 min absorption spectra were taken. The absorption spectrum of the product against reagent blank is shown in Figure 1. 510 nm is the maximum absorption wavelength for both solutions. Colourless reagent blank had practically negligible absorbance at this wavelength.

Optimisation of the variables

To study the effect of the concentration of NQS and NaOH on the maximum absorbance, a number of preliminary experiments were carried out. In a series of 25 mL volumetric flasks containing 50 μg mL^{-1} of the drug solution, keeping one reagent amount as constant, the concentration of the other reagent was varied and the mixture was diluted up to the mark with water. After 15 min absorbance of each solution was measured at 510 nm. It was found that a 0.05% solution of NQS in the range 3.0 to 4.0 mL and 0.5 M NaOH in the range 4.5 to5.5 mL were necessary to achieve maximum colour intensity. Therefore, 3.5 mL of

NQS and 5 mL of NaOH were recommended for all measurements. Results obtained for the optimisation of the variables are presented graphically in Figure 2.

The effect of time on maximum absorbance was also tested by measuring the absorbance of both the solutions at regular intervals and it was found that solutions show maximum absorbance after 15 min and are stable for a further 24 h.

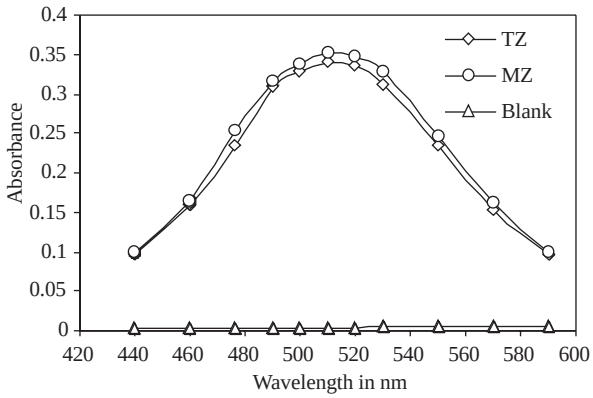


Figure 1. Absorption maximum of TZ and MZ against blank.

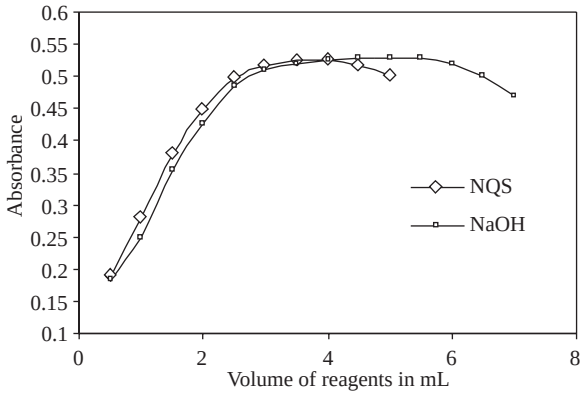
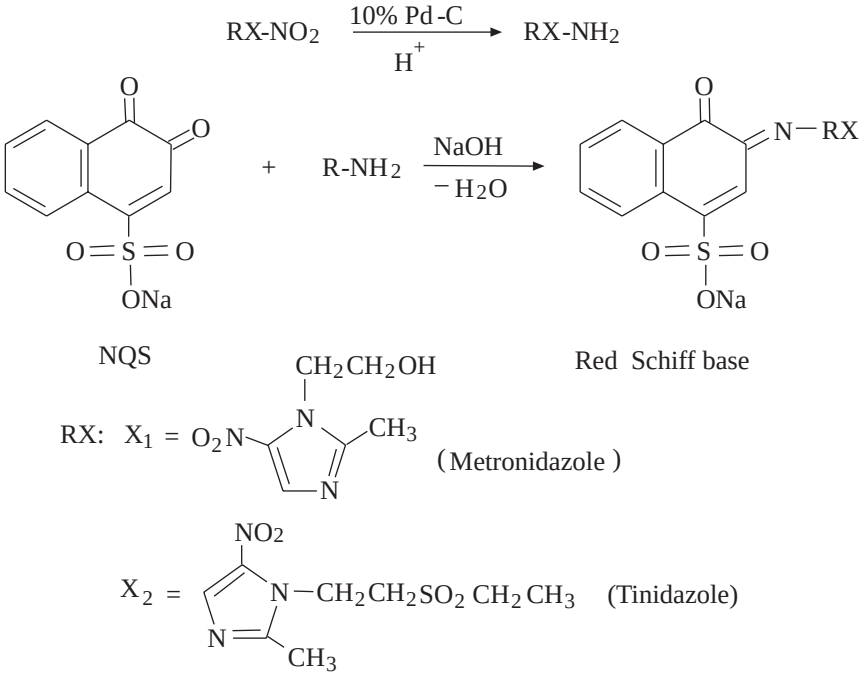


Figure 2. Effect of reagent concentration on absorbance.

Reaction sequence

Reduction of the nitro groups of both the drugs was carried out as explained in the experimental section. When the resulting amine undergoes a condensation reaction in alkaline medium with NQS it forms a red Schiff base with maximum absorbance at 510 nm. Although the reaction between the reduced drug and NQS is instantaneous, for the development of maximum colour, the reaction mixture has to be kept for a further 15 min. The probable reaction mechanism based on the reported method[26]is given below.



Optical characteristics and validation of the method

Optical characteristics such as Beer's law limits, molar absorptivity and Sandell's sensitivity, for both tinidazole and metronidazole, are given in Table 1. Data of the regression analysis using the least squares method made for the calibration curves are also given in the same table. The accuracy and precision of the method were checked by analysing 6 replicate samples within the Beer's law range containing the same amount of each drug. Values of RSD were below 0.9%. The lower values of RSD indicate the good precision and reproducibility of the method.

The validity of the proposed procedure for the determination of tinidazole and metronidazole in their pure state was checked by analysing these drugs using the proposed method. The results obtained for pure drugs were reproducible with low (0.81 and 0.33) relative standard deviations (RSD).

The limit of quantification (LOQ) was determined by taking the ratio of the standard deviation (σ) of the blank with respect to water and the slope of the calibration curve multiplied by the factor 10. This means that LOQ is approximately 3.3 times greater than LOD. LOD is well below the lower limit of the Beer's law range.

Interference studies

To study the potential interference problems from the commonly used excipients and other additives such as glucose, dextrose, lactose, starch, sodium alginate, talc, magnesium alginate, magnesium stearate, and ascorbic acid, recovery studies were carried out. Under the experimental conditions employed, to a known amount of drug (metronidazole and tinidazole 10 μg mL^{-1}), excipients in different concentrations were added and analysed. Results of the recovery analysis are presented in Table 2. Excipients up to the concentrations shown in the Table do not interfere with the assay. In addition recoveries in most cases were 100% and the lower values of the RSD indicate the good precision of the method.

Table 1. Optical characteristics and validation data.

Parameters	tinidazole	metronidazole
λ max (nm)	510	510
Beer's law limit (μg mL^{-1})	2 – 45	1.5 – 37
Molar absorptivity (L mol^{-1} cm^{-1})	8.662 x 10^3	7.893 x 10^3
Sandell's sensitivity (μg cm^{-2})	0.0285	0.0216
Stability (h)	24 hr	24 hr
Regression equation (y)*		
Slope, b	0.0312	0.0345
Intercept, a	0.002	0.005
Correlation coefficient, r	0.9998	0.9999
Recovery data ± RSD (%)**	9.89 ± 0.81	10.24 ± 0.33
Range of error, % ± (at 95% confidence limit)	0.89	1.12
Limit of detection (μg mL^{-1})	0.44	0.36
Limit of quantification (μg mL^{-1})	1.35	1.10

* Y = a + bx, where x is the concentration in μg mL^{-1}

** Six replicate analysis of 10 μg mL^{-1} of the drugs.

Table 2. Determination of metronidazole and tinidazole[a] in the presence of excipients.

Excipients	Amount taken (mg mL^{-1})	% Recovery ± % RSD[b] Metronidazole	Tinidazole
Glucose	48	98.9 ± 0.82	99.21 ± 0.56
Starch	55	101.2 ± 0.65	98.38 ± 0.34
Lactose	40	100.02 ± 0.48	101.08 ± 0.54
Talc	27	98.9.5 ± 0.58	99.12 ± 0.39
Dextrose	53	100.4 ± 0.67	100.28 ± 0.43
Stearic acid	36	99.3 ± 0.88	100.23 ± 1.02
Sodium alginate	29	100.2 ± 0.75	101.08 ± 0.65
Magnesium alginate	32	101.04 ± 0.90	100.84 ± 0.74
Magnesium stearate	35	100.3 ± 0.85	101.24 ± 1.04
Ascorbic acid	42	100.6 ± 0.64	99.64 ± 0.82

[a] 10 μg mL^{-1} of metronidazole and tinidazole were used.

[b] Average of 6 replicate analyses.

Applicability of the method

The applicability of the proposed spectrophotometric procedure for the assay of tinidazole and metronidazole was tested by analysing various available commercial formulations. The samples were also analysed using the official method. The results (Table 3) of the analysis showed that the data are consistent with the label claim of the formulations. The calibration curves showed a linear response over the range of concentrations used in the assay procedures. The RSD values in the range 1.05 to 0.38 for the reproducibility and recovery studies show that the method is precise and accurate. The precision and accuracy of the method was further compared statistically using Student's t test and variance ratio F test. At a 95% confidence level, the calculated t-values and F-values do not exceed the tabulated values. Optical characteristics, precision and accuracy data are presented in Table 3.

Table 3. Analysis of tinidazole and metronidazole formulations.

Commercial formulations analysed	Drug content	Label claim in mg	Amount of drug found in mg*		t value	F value
			Developed method	Official method (IP)		
Tablets						
Tiniba[a]	TZ	300	299.83 ± 0.56	298.95 ± 1.02	0.98	1.81
Fasagyn[b]	TZ	500	499.02 ± 0.76	498.12 ± 0.95	0.76	1.24
Tina300[c]	TZ	300	300.16 ± 0.38	302.34 ± 1.23	1.26	3.26
Metrogyl[d]	MZ	400	399.02 ± 1.04	398.10 ± 0.98	0.54	1.06
Flagyl[e]	MZ	400	401.03 ± 0.56	403.08 ± 0.86	0.84	1.54
Aristogyl[f]	MZ	200	200.23 ± 0.47	198.44 ± 0.76	0.89	1.60
Injections						
Metrogyl[g]	MZ	500 mg /100 mL	501.28 ± 1.05	502.18 ± 1.10	0.68	1.04
Metron[h]	MZ	500 mg /100 mL	498.98 ± 0.73	498.04 ± 0.86	0.63	1.17
Suspension						
Metrogyl[l]	MZ	200 mg /5 mL	200.76 ± 0.70	201.72 ± 1.18	0.93	1.69

*Average of 6 determinations ± RSD

t values (n = 5, at 95% confidence level tabulated value 2.57)

F values (n_1 and n_2 = 5, at 95% confidence level tabulated value 5.05)

Marketed by Zydus Cadila[a], Pfizer[b], Bombay tablets[c], Unique[d,g,I], Rhone-Poulenc[e], Aristo[f], Alkem[h]

In addition it is observed that there is no interference from the excipients used in the formulations. Hence, this method can be adopted for the routine quality control of tinidazole and metronidazole in bulk as well as in formulations.

Conclusion

Reported spectrophotometric methods for the assay of these drugs are based on the initial reduction of the nitro group of drugs using the Zn-HCl reduction system and it was observed that all those methods suffer from low sensitivity and that some methods involve additional tedious procedures such as diazotisation, heating or extraction. A comparison of the present method with the reported methods is given in Table 4. The novel reduction system proposed in this method comprises 10% Pd-C and formic acid and gives a direct condensation reaction with NQS to form red Schiff base. In addition the advantages of this reduction mixture are highlighted in the experimental section. Interestingly, the sensitivity of the method surpasses that of the reported methods within a wide range of detection limits. The proposed spectrophotometric method is simple, sensitive, accurate and rapid.

Table 4. Literature survey of the spectrophotometric determination of tinidazole and metronidazole.

Reagents used	λmax in nm	Beer's law range in μg mL^{-1}	Critical experimental conditions involved	Refer ence
p-Dimethyl amino cinnam aldehyde	510	50 – 400 for MZ	Involves reduction with Zn-HCl and low sensitivity. Analysed only MZ.	14
4-Dimethyl amino benzaldehyde	550	10 – 100 for TZ	Involves reduction with Zn-HCl and low sensitivity. Analysed only TZ.	15
β-Naphthol	480	10 – 80 for MZ	Involves reduction with Zn-HCl and diazotisation and coupling with the cited reagent. Low sensitivity. Analysed only MZ.	16
Metol and $K_2Cr_2O_7$	720	2.4 – 24 for TZ 1.6 – 16 for MZ	Involves reduction with Zn-HCl and the use of buffer of pH 2.9 and colour formation, and its stability is pH dependent.	17
NN-dimethyl-p-phenylenediamine and chloramine-T	540	4 – 36 for TZ 3 – 24 for MZ	Involves reduction with Zn-HCl and the use of buffer of pH 7 and colour formation and its stability is pH dependent.	18
Vanillin	412	10 – 50 for TZ	Involves reduction with Zn-HCl and heating for20 min with the reagent and cooling before absorbance measurement. Analysed only TZ.	19
Salicylaldehyde	380	20 – 70 for TZ	Involves reduction with Zn-HCl and low sensitivity. Analysed only TZ.	20
Bromocresol purple	618	2 – 24 for MZ	Involves extraction with $CHCl_3$ and use of buffer of pH 10.	21
Bromocresol green	654	2 – 22 for MZ	Involves extraction with $CHCl_3$ and use of buffer of pH 9.5.	-do-
NaOH and KCl	368	10 – 30 for TZ	Low sensitivity and involves heating at 100°C for 10 min.	22
Bromothymol blue	440	not given	Involves extraction with $CHCl_3$ and use of buffer of pH 4.4.	23
Methylbenzothiazolin-2-onehydrazine (MBTH)	500 and 490	1-32 for MZ 4-36 for TZ	Involves reduction with Zn-HCl and MBTH is a costly reagent.	24
N(1-naphthyl) ethylene diamine dihydrochloride (NEDA)	520 and 505	0.5 – 18 for MZ and TZ	Involves reduction with Zn-HCl and an additional step of diazotisation. Beer's law valid for low range of concentration.	24

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References

1. M. E. Wolff, '**Burgers Medicinal Chemistry**' 4^{th} Edition, John Wiley and Sons, New York, 428-429 (1979).
2. C. Cosar and L. Julon, **Ann. Inst. Pasteur 96**,238 (1959).
3. C. Cosar, P. Ganter and L. Julion, **Presse Meed 69**, 1069 (1961).
4. C. Cosar, C. Cusan, R. Horclois, R.M. Jacob, J. Robert, S. Tchelitcheff and R. Vaupre, **Arzneim -Forsch 16,** 23-29 (1966**)**.
5. S.J. Powell, I. McLeod, A.J. Wilmot and R. Elsdon-Dew, **Lancet**, **2,** 1329 (1966).
6. S.J. Powell, **Bull. N.Y. Acad. Med. 47,** 469-470, (1971).
7. R.B. Khambatta, **Ann. Trop. Med. Parasitol. 62,** 139 (1968).
8. M.W. Miller, H.L. Howes, Jr., R.V. Kasubick and A.R. English, **J. Med. Chem. 13,** 849 (1970).
9. P.R. Sawyer, R.N. Brogden, R.M. Pinder, T.M. Speight and G.S. Avery, **Drugs**, **11,** 423 (1976).
10. **Remington's Pharmaceutical Sciences,** 18^{th} Edition., Mack Publishng Co., Easton, 1228-1230 (1993).
11. **Indian Pharmacopoeia**, Controller of Publications, New Delhi, **1,** pp. 488 and **2,** pp. 764 (1996).
12. **British Pharmacopoeia**, HM Stationery Office, London, pp. 98, 893,1814 and 1815 (1998).
13. **United States Pharmacopoeia** – **24,** NF 19, US- Pharmacopoeial Convention, Rockville, MD, 1104 –1106 (2000).
14. B.A. Moussa, **Int. J. Pharm. 10,** 199-207 (1982).
15. O.S. Kamalapurkar and J.J. Chudasama, **East Pharm. 26,** 207-208 (1983).
16. T.P. Gandhi, P.R. Patel, V.C. Patel and S.K. Patel, **J. Inst. Chem. 56,** 127-128 (1984).
17. C.S.P. Sastry, M. Aruna and A.R.M. Rao, **Talanta**,**35,** 23-25 (1988).
18. C.S.P. Sastry, M. Aruna, A.R.M. Rao and A.S.R.P. Tipirneni, **Chem. Anal. (Warsaw), 36,** 153-158 (1991).
19. N.M. Sanghavi, N.G. Joshi and D.G. Saoji, **Indian J. Pharm. Sci., 41,** 226-228(1979).
20. O.S. Kamalapurkar and C. Menezes, **Indian Drugs**,**22,** 164 (1984).
21. A.S. Amin, **Anal. Lett, 30,** 2503-2513 (1997).
22. M.L. Lopez, F.J.L. Vazquez and P.L. Lopez-de-Alba, **Anal. Chim. Acta,** 340, 241-244 (1997).
23. R.G. Bhatkar and S.K. Chodankar, **Indian J. Pharm. Sci. 42,** 127-129 (1980).
24. P. Nagaraja, K.R. Sunitha, R.A. Vasantha and H.S. Yathirajan, **J. Pharm. Biomed. Anal., 28,** 527-535 (2002).
25. D. Channe Gowda and Shankare Gowda, **Ind. J. Chem. 39B,** 709-711 (2000).
26. P. Nagaraja, K.C. Srinivasa Murthy and H.S. Yathirajan, **Talanta, 43,** 1075-1080 (1996).