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Stability Indicating HPLC Method for the Determination of Hydrochlorothiazide in Pharmaceutical Dosage form

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ABSTRACT

A stability indicating analytical method has been developed and validated. In this study Hydrochlorothiazide was degraded under different stress test conditions as per International Conference on Harmonization. The degraded samples were used to develop a stability-indicating high performance liquid chromatographic (HPLC) method for the Hydrochlorothiazide. The Hydrochlorothiazide was well separated from degradation products using a reversed-phase (C-18) column and a mobile phase comprising of Methanol: Buffer pH- 3.2 (60:40 v/v) and other HPLC parameters were flow rate 1 mL/min, detection wavelength 270 nm and injection volume 20 µl. The method was validated for linearity, precision, accuracy, ruggedness and robustness. Results obtained after validation study indicating that the proposed single method allowed analysis of Hydrochlorothiazide in the presence of their degradation products formed under a variety of stress conditions. The developed procedure was also applicable to the determination of stability of the Hydrochlorothiazide in commercial pharmaceutical dosage form.

INTRODUCTION

Hydrochlorothiazide (Goodman et al, 1996; Wankhede et al, 2007) is thiazide diuretic mainly used for the treatment of hypertension. Chemically Hydrochlorothiazide is, 6-Chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7- sulfonamide -1,1-dioxide. Figure 1.

There are few analytical methods available for its estimation in biological fluids includes HPLC and UV detection (Erk et al, 2003; Shah et al, 2006) since there is need to develop the stability indicating method which will show the degradation behavior of the Hydrochlorothiazide.

The aim of the present study was to develop an accurate, precise, specific, reproducible and stability indicating HPLC method for the estimation Hydrochlorothiazide. The Hydrochlorothiazide was purposely degraded by acid, base, oxide, dry heat, wet heat and UV light treatment to check the stability and to develop stability indicating assay method (Bakshi et al, 2004; Kaul et al, 2004).

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The developed analytical method was validated by means of linearity, accuracy, precision, LOD, LOQ, ruggedness and robustness as per International Conference on Harmonization (ICH) guidelines (USP 2000; ICH 2003).

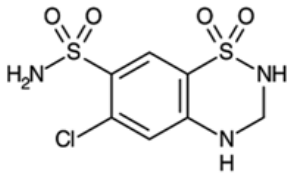

Fig. 1: Structure of Hydrochlorothiazide.

MATERIALS AND METHODS

Hydrochlorothiazide was supplied by Dr. Reddy's Laboratory, Hyderabad. All the chemicals used during the study were of AR / HPLC / GC grades and procured from Merck, Mumbai, Qualigens, Mumbai, Rankem, New Delhi, etc. Millipore membrane filters (0.45µ) were used for filtration of mobile phase and working solutions. Double distilled water and the volumetric glasswares of class 'A' grade were used throughout the experimental work.

Shimadzu HPLC system consists of following components:

Shimadzu HPLC pump LC-10ATvp, On Line Degasser DGU-14A, Low pressure Gradient flow control unit FCV-10ALvp, Rheodyne injector 7725 I with 20 µl loop, Column oven CTO-10ASvp, UV-VIS detector SPD-10AVp with SHIMADZU class CSW software, Column Thermo Hypersil ODS 5 µ C18 (250 x 4.6mm)

Forced Degradation of Hydrochlorothiazide

Acid induced degradation

The 50 mg of Hydrochlorothiazide was dissolved in 20 mL of methanol; 5 mL of 1N Hydrochloric acid (HCl) was added. The solution was refluxed for 6.0 h at 96-98°C on boiling water bath. After cooling at room temperature acid was neutralized with 1 N NaOH then volume was completed up to 50 mL with methanol to obtain concentration 1.0 mg/mL.

Base induced degradation

The 50 mg of Hydrochlorothiazide was dissolved in 20 mL of methanol; 5 mL of 1N NaOH was added. The solution was refluxed for 6.0 h at 96-98°C on boiling water bath. After cooling at room temperature base was neutralized with 1 N HCl then volume was completed up to 50 mL with methanol to obtain concentration 1.0 mg/mL.

Hydrogen peroxide induced degradation

The 50 mg of Hydrochlorothiazide was dissolved in 20mL of methanol; 5 mL of 30 % Hydrogen peroxide was added. The solution was kept for 6.0 h in dark at room temperature then the volume was completed up to 50 mL with methanol to obtain concentration 1.0 mg/mL. 6.0 h in dark at room temperature then the volume was completed up to 50 mL with methanol to obtain concentration 1.0 mg/mL.

Wet heat degradation

The 50 mg of Hydrochlorothiazide was dissolved in 20 mL of methanol; 5 mL of water was added. The solution was refluxed for 6.0 h at 96-98°C on boiling water bath. After cooling at room temperature the volume was completed up to 50 mL with methanol to obtain concentration 1.0 mg/mL.

Dry heat degradation

The Hydrochlorothiazide was kept in oven at 110 ºC for 12 h, 50mg of drug was weighed and dissolved in methanol to obtain concentration 1.0 mg/mL to study dry heat degradation.

Photochemical degradation

The Hydrochlorothiazide drug was kept in sun light for 30 h (cumulative), 50mg of drug was weighed and dissolved in methanol to obtain concentration 1.0 mg/mL to study photochemical degradation. All six degraded samples solutions of concentration of 1mg/mL from the solution 5mL was pipette out and diluted up to 10 mL with methanol to obtain 500µg/mL, from the solution 5mL was pipette out and diluted up to 25 mL with mobile phase to obtain concentration 100µg/mL which was used for degradation study.

Development of HPLC method:

HPLC studies were carried out on all the reaction solutions individually, and on a mixture of the solutions in which degradation was observed. The separations were achieved by isocratic elution using Methanol: Buffer (60:40 v/v) [Buffer Preparation - 2.7gm of KH_2PO_4 was dissolved in 1000ml of water and pH -3.2 adjusted with Ortho-Phosphoric acid] as the mobile phase. It was filtered through 0.45 µ membrane filter and degassed before use. The injection volume was 20 µL. The working concentration was 100µg/mL and mobile phase flow rate was 1 mL/min on C18 column with column oven temperature 30°C. The detection was carried out at 270 nm. Chromatogram of Hydrochlorothiazide is shown in Figure 2.

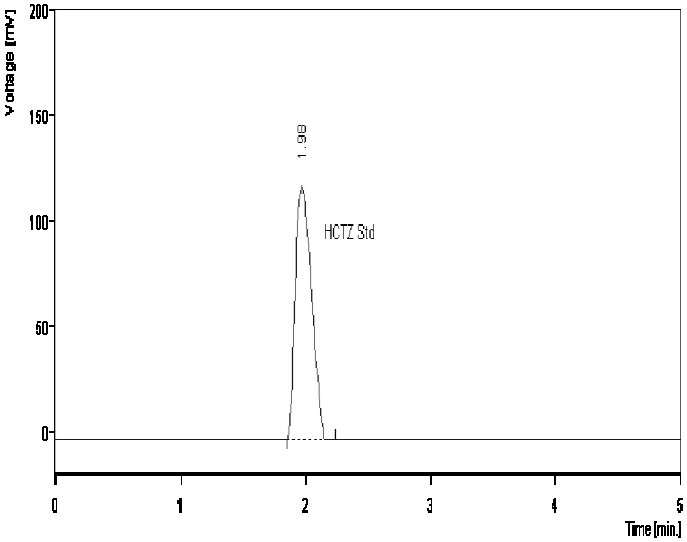

Fig. 2: Chromatogram of Hydrochlorothiazide standard.

Validation Parameters

Accuracy

Accuracy of proposed method was ascertained on the basis of recovery studies performed by standard addition method. In the study standard was added to fixed quantity of tablet powder at different levels of labeled claim (i.e. 60 to 140 % of labeled claim).

Precision

Precision of any analytical method was ascertained by replicate estimation of drug in sample of homogeneous tablet powder by different analyst, intraday and interday. The results are expressed as SD and % RSD of series of measurements.

Limit of Quantitation (LOQ) and Limit of Detection

The quantitation limit was determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

Linearity and range

The linearity of analytical method was determined by injecting 50 to 200 µg/mL of Hydrochlorothiazide and peaks were recorded and plotted graph, concentration versus area response of Hydrochlorothiazide. The range of analytical method was established with a suitable level of precision, accuracy and linearity.

Application of developed method on marketed formulation

Standard Preparation

Accurately weighed quantity of Hydrochlorothiazide (25 mg) was dissolved in the methanol and volume was completed up to the 50 mL to obtain conc 500µg/mL, 5mL of the solution was diluted up to 25 mL with mobile phase to obtain conc 100µg/mL.

Sample Preparation

Twenty tablets were weighed and finely powdered. An accurately weighed quantity of tablet powder equivalent to 25 mg of Hydrochlorothiazide was taken into 50 mL volumetric flask and dissolved in the methanol. It was then sonicated for 10 min and volume was completed to the mark with the methanol. The solution was filtered through 0.45µ membrane filter. 5 mL of the filtered solution diluted to 25 mL with mobile phase to obtain conc 100 µg/mL of Hydrochlorothiazide. The percent label claim was calculated against the standard.

RESULT AND DISCUSSION

HPLC studies of samples obtained on stress testing of Hydrochlorothiazide under different conditions using Methanol: Buffer (60:40 v/v) as the mobile solvent system suggested the following degradation behavior.

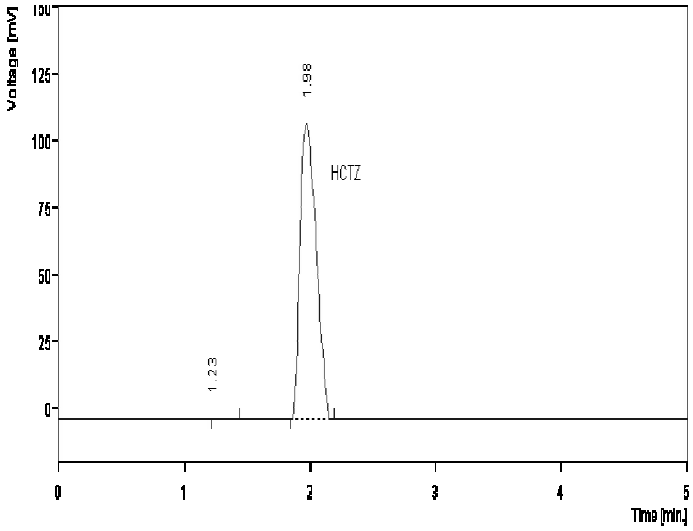

Fig. 3: Chromatogram of Hydrochlorothiazide and its acid degradation products.

Acid Induced Degradation Product

The chromatogram of the acid degraded sample for Hydrochlorothiazide showed additional peak at RT 1.23 min (Figure 3) indicating that Hydrochlorothiazide undergoes degradation under acidic condition.

Base Induced Degradation Product

The chromatogram of the base degraded sample for Hydrochlorothiazide showed additional peak at RT value of 1.58 min. (Figure 4) indicating that Hydrochlorothiazide undergoes degradation under basic condition.

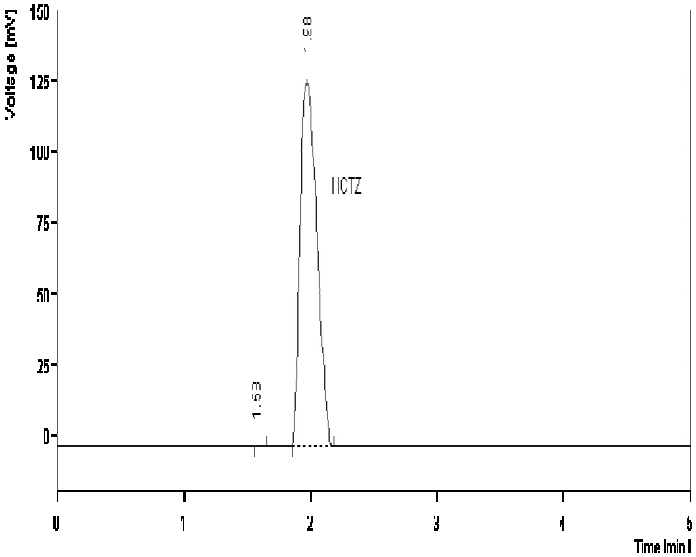

Fig. 4: Chromatogram of Hydrochlorothiazide and its base degradation products.

Hydrogen Peroxide Induced Degradation Product

The chromatogram of the hydrogen peroxide degraded sample for Hydrochlorothiazide showed additional peak at RT value of 1.46 min and 3.07 min (Figure 5) indicating that Hydrochlorothiazide undergoes degradation under hydrogen peroxide condition.

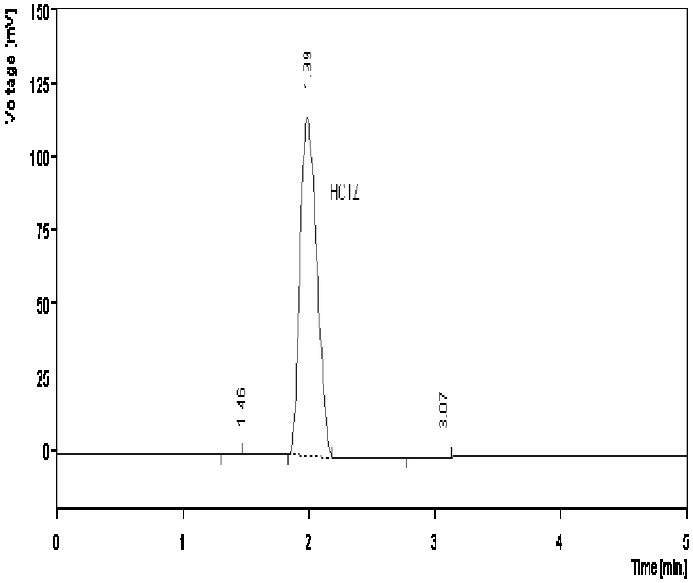

Fig. 5: Chromatogram of Hydrochlorothiazide and its oxide degradation products.

Wet heat Degradation Product

The wet heat degraded samples of Hydrochlorothiazide did not show extra peak (Figure 6). The samples showed degradation within 2% of Hydrochlorothiazide.

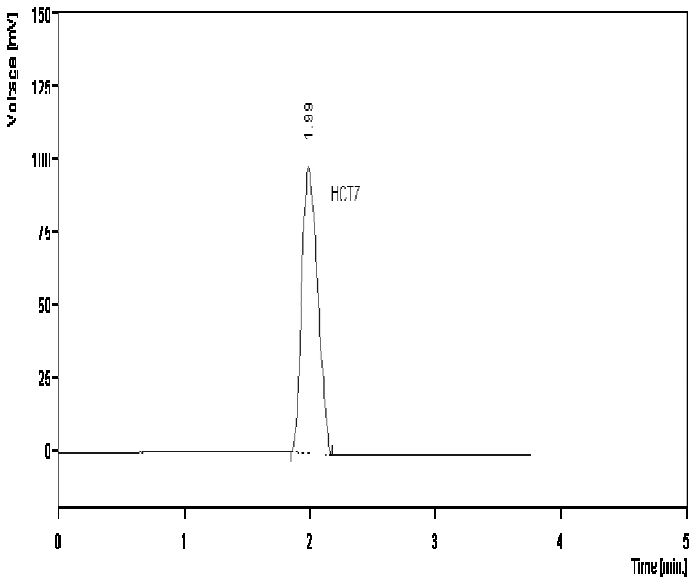

Fig. 6: Chromatogram of Hydrochlorothiazide and its wet heat degradation products.

Dry Heat Degradation Product

The dry heat degraded samples of Hydrochlorothiazide did not show extra peak (Figure 7). The samples showed degradation within 2% of Hydrochlorothiazide.

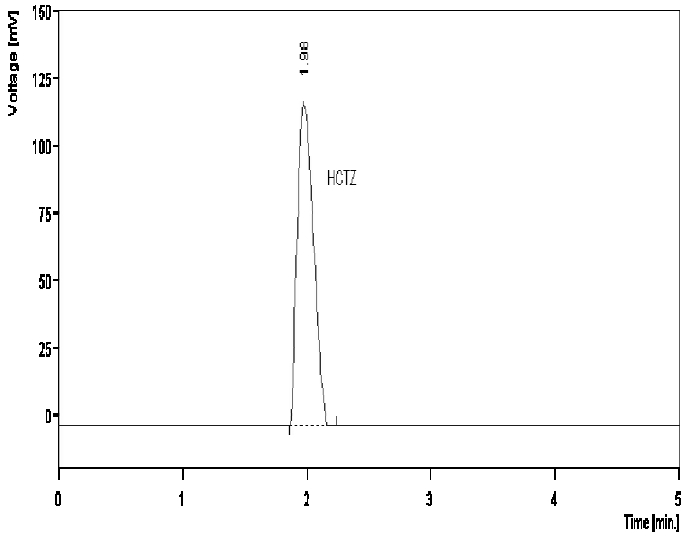

Fig. 7: Chromatogram of Hydrochlorothiazide and its dry heat degradation products.

Photochemical Degradation Product

The photo degraded sample showed small additional peak at RT 1.19 min (Figure 8) indicating that Hydrochlorothiazide undergoes degradation under photochemical exposure. The forced degradation study was used to develop stability indicating analytical method for Hydrochlorothiazide and it was also used to study the stability of the Hydrochlorothiazide. The results of forced degradation study are shown in Table 1.

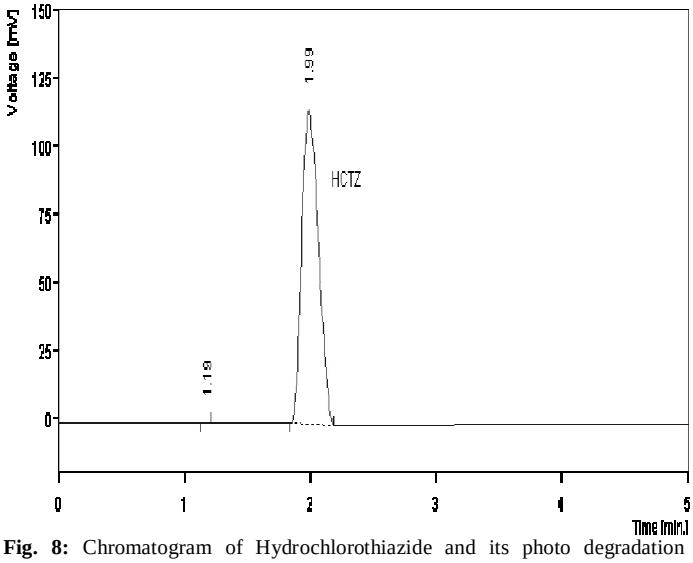

Fig. 8: Chromatogram of Hydrochlorothiazide and its photo degradation products.

Table. 1: Results of Degradation Study.

Sr. No.	Treatment	Time (h)	% of HCTZ *		% of Degradation*
			Assay	±SD	
1.	Refluxed with 5 ml of 1N HCl on boiling water bath	6	96.56	0.8556	3.44
2.	Refluxed with 5 ml of 1N NaOH on boiling water bath	6	94.65	0.4565	5.35
3.	Stored with 5 ml of 30% H_2O_2 in dark at room temperature	6	93.42	0.2545	6.58
4.	Refluxed with 5 ml water on boiling water bath	6	98.12	0.6525	1.88
5.	Dry Heat 120°C	12	98.45	0.8525	1.55
6.	Photo degradation	30	97.85	0.5645	2.15

** Each value is mean of five observations*

Method Validation

Accuracy

The HPLC area responses for accuracy determination are depicted in Table 2. Good recoveries (100.51–101.21%) of the spiked drug were obtained at each added concentration, indicating that the method was accurate.

Table. 2: Results of Recovery Study.

Aquazide Tablet (Avg. Wt. 101.1 mg for 12.5 mg of HCTZ)

Sr. No.	Wt. of Tablet (mg)	Amount of Pure drug added (mg)	Peak area of standard	Peak area of sample	% Recovery*
1	101.15	2.5	1054.25	635.45	100.51
2	101.56	7.5	1054.25	845.52	100.71
3	101.58	12.5	1054.25	1060.25	101.05
4	101.36	17.5	1054.25	1275.85	101.11
5	101.89	22.5	1054.25	1482.23	101.21
				Mean	100.92
				±S.D.	0.2961
				R.S.D.	0.2934

** Each value is mean of five observations*

Precision

Results of precision study are shown in Table 3 and Table 4. The values of SD and RSD under different conditions are within the prescribed limit of 2 %, showing high precision of the method.

Table. 3: Results of Precision Study (Different Analyst).

Sr. No.	Different analyst	% Drug estimation*
		Aquazide
1	I	101.56
2	II	100.58
3	III	98.56
	Mean	100.23
	±S.D.	1.5297
	R.S.D.	1.5262

** Each value is mean of five observations*

Table. 4: Results of Precision Studies. (Intraday And Interday)

Sr. No.		% Drug Estimation*	
		Aquazide	
		Intraday	Interday
1	I	99.56	98.99
2	II	100.98	101.25
	Mean	100.27	100.12
	±S.D.	1.0041	1.5981
	R.S.D.	1.0014	1.5961

** Each value is mean of five observations*

Limit of Quantitation and Limit of Detection

Developed stability indicating method was able to determine limit of quantitation 15µg/mL minimum level concentration of hydrochlorothiazide with high accuracy and precision and limit of detection is 10µg/mL.

Linearity and range

The response for the drug was linear ($r2 = 0.9990$) in the concentration range between 60-140 µg/mL.

Application of developed method on marketed formulation

The developed HPLC method is suitable for estimation of Hydrochlorothiazide as it extracts the drug from formulation and determines the percent content with good %RSD. The results of the application of developed method are shown in Table 5.

Table. 5: Results of Estimation of Hydrochlorothiazide in Tablets.

Aquazide (Avg. Wt. 101.1 mg for 12.5 mg of HCTZ)

Sr. No.	Weight of Sample (mg)	Peak area of standard	Peak area of sample	% Drug Estimation*
1	201.56	1054.25	1060.25	100.89
2	202.58	1054.25	1054.86	99.87
3	201.78	1054.25	1078.56	102.52
4	204.56	1054.25	1056.56	99.06
5	204.12	1054.25	1045.96	98.28
			Mean	100.12
			±S.D.	1.6514
			R.S.D.	1.6494

**Each value is mean of five observations*

CONCLUSION

The developed HPLC technique is precise, specific, accurate and stability indicating. The developed method was validated based on ICH guidelines. Statistical analysis proves that the method is repeatable and selective for the analysis of Hydrochlorothiazide as bulk drug and in pharmaceutical formulations.

The method can be used to determine the purity of the drug available from the various sources by detecting the related impurities. It may be extended to study the degradation kinetics of Hydrochlorothiazide and for estimation of pure and its metabolites in plasma and other biological fluids.

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REFERENCES

Bakshi M. Singh S. HPLC and LC-MS Studies on Stress Degradation Behavior of Tinidazole and Development of a Validated Specific, Stability Indicating HPLC Assay Method. J Pharm Biomed Anal 2004; 34: 11–18.

Goodman and Gilman's, in: A.G. Gilman, T.W. Rall, A.S. Nies, P. Taylor (Eds.), the Pharmacological Basis of Therapeutics. Pergamon Press, Oxford, 1996

International Conference on Harmonization**,** Draft Guidelines on Validation of Analytical Procedures, Definitions and Terminology, 'Federal Register', 2000.

International Conference on Harmonization, Q1A9 (R2) Stability Testing of New Drug and Product, step 5 version, 2003.

Kaul N, Agrawal H, Paradkar AR, Mahadik KR: HPTLC Method for Determination of Nevirapine in Pharmaceutical Dosage Form. Talanta 2004; 62: 843–852.

Nevin Erk., "Simultaneous Determination of Irbesartan and Hydrochlorothiazide in Human Plasma by Liquid Chromatography", J. Chromatogr. B, 2003, 784(1) 195-201.

S.B. Wankhede, M.R. Tajne, K.R. Gupta, S.G. Wadodkar. Ind. J. Pharm. Sci. 2007, 69, 298-300.

Shah, H.J., Kataria, N. B., Subbaiah G. and. Patel, C. N., "Simultaneous LC-MS-MS analysis of Valsartan and Hydrochlorothiazide in Human plasma", Chromatogr., 2006, 69(9-10), 1055-1060.

The United States Pharmacopoeia, 24/ National Formulary 19, The United States Pharmacopoeial Convection, Rockville, 2000; 1920-24.

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