Journal of Chromatography, 190 (1980) 429–435

CHROM. 12,525

PURITY DETERMINATION OF ZOMEPIRAC SODIUM DIHYDRATE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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(Received November 6th, 1979)

SUMMARY

A high-performance liquid chromatographic method has been developed to determine the purity of zomepirac sodium dihydrate, a novel non-narcotic analgesic agent. This method is sensitive, reproducible, and accurate. The calibration curves are linear over the range of interest for all the related compounds and impurities. A minimum of 0.1% impurity can be easily detected and quantitated.

INTRODUCTION

In 1973, Carson and Wong[1] reported on the synthesis of several benzoyl pyrrole acetic acids. One of these, Zomax® (tradename of McNeil Labs., Fort Washington, Pa., U.S.A.), zomepirac sodium dihydrate (sodium 5-(4-chlorobenzoyl)-1,4-dimethyl-1*H*-pyrrole-2-acetate dihydrate) was found to be a potent non-narcotic analgesic, shown effective in clinical studies[2–4].

A high-performance liquid chromatographic analytical method was developed for drug purity and stability determinations. The method can separate and quantitatively determine the synthetic impurities and degradation products from the drug substance. In addition, it does not generate any impurities or degradation products.

The method requires a simple dissolution of the drug in a solvent and a direct injection into an HPLC column. Zomepirac sodium dihydrate absorbs strongly at 254 nm, making it an ideal candidate for UV detection. Minimum sample preparation is required, making it possible to reduce sample preparation error and to increase the accuracy of analysis. In addition, at ambient temperature, potential problems of reactivity between zomepirac sodium dihydrate and known or unknown impurities are minimized.

The chromatographic conditions are described in this paper for the separation and quantitation of 16 related compounds and impurities of zomepirac sodium dihydrate. Some of the impurities were identified in the drug substance by infrared, nuclear magnetic resonance and mass spectrometry. The method is also applicable for stability testing, purity analysis, and dosage form stability. During the course of the investigation, a synthetic impurity which did not elute from the silica gel column

was discovered. A reversed-phase chromatographic method was therefore developed in conjunction with the normal-phase chromatographic method.

EXPERIMENTAL

Equipment

A Waters Assoc. Model ALC-202 liquid chromatograph, equipped with a UV detector (254 nm), dual 6000 p.s.i. pumps, a Model U6K injector, and a Model 660 solvent programmer, was used throughout the study. The output of the UV detector was connected to a recorder and a Hewlett-Packard Model 3352B laboratory data system.

Columns

LiChrosorb® Si 60 (10 μm silica gel) was obtained from EM Labs., (Elmsford, N.Y., U.S.A.) and was slurry-packed under high pressure, using a Haskel air-driven fluid pump (Haskel Engineering and Supply Co., Burbank, Calif., U.S.A.), into a 25 cm × 2.1 mm I.D. stainless-steel column, as previously described[5].

LiChrosorb RP-18 reversed-phase packing (10 μm silica gel chemically bonded with octadecylsilane obtained from EM Labs.) was slurry-packed under high pressure into a 25 cm × 3.2 mm I.D. stainless-steel column. The procedure for packing the reversed-phase column was basically the same as that described for packing a silica gel column, except that the packing was not dried and water, instead of hexane, was used as the mobile phase.

Reagents

Methylene chloride, *n*-hexane and propan-2-ol, distilled in glass, were obtained from Burdick & Jackson Labs., Muskegon, Mich., U.S.A. The glacial acetic acid was ACS grade, and the methanol and ammonium hydroxide were analytical reagent grade from Mallinckrodt, St. Louis, Mo., U.S.A. The distilled water, the zomepirac sodium dihydrate and various impurities and degradation products were obtained from McNeil Labs.

Method

The micro silica gel column was placed in the chromatograph. Solvent reservoir A was filled with 0.25% acetic acid in *n*-hexane and solvent reservoir B was filled with 0.25% acetic acid and 10% propan-2-ol in *n*-hexane. The column flow-rate and the UV detector were set at 2.0 ml/min and 0.10 a.u.f.s., respectively. The column was conditioned with 60 ml of mobile phase from reservoir B.

A 20-mg amount of zomepirac sodium dihydrate was dissolved in 1.0 ml of methanol–methylene chloride (9:1) solvent. A 5.0-μl aliquot of this solution was injected into the chromatograph. A concave gradient, No. 7 setting, was used from 3% to 90% mobile phase B over 20 min.

The reversed-phase column was next placed into the chromatograph. The pump reservoir was filled with 0.03% ammonium hydroxide, 19.97% methanol, and 80.00% water. The flow-rate and the UV detector were set at 2.0 ml/min and 0.05 a.u.f.s., respectively. The column was conditioned by passing through 60 ml of

mobile phase. A 5.0-μl aliquot of the sample (100 μg) was injected into the chromatograph.

Calibration curve preparation

A calibration curve was prepared as follows: 1.0 mg of a particular test compound or standard was dissolved in and diluted to 10 ml with methylene chloride or methanol. A 10:1 diluted solution was also prepared. Several aliquots of solution (corresponding to various concentrations) were injected onto the HPLC column. A calibration curve was then prepared by plotting the observed peak area against the amount of sample injected.

Reproducibility of retention times

Zomepirac sodium dihydrate, some of its possible impurities, and several related compounds were mixed and dissolved in methanol. A 5.0-μl aliquot of this mixture was injected into the chromatograph equipped with the silica gel column. Multiple injections were made.

Recovery study

A 20-mg amount of zomepirac sodium dihydrate reference standard was dissolved in 1.0 ml of a methanol solution containing known amounts of compounds 2, 7, 8, 9, and 12 (Table I). A 5-μl volume of this mixture was injected onto the silica gel column. Recovery was determined from the calibration curves, which were prepared as described above.

RESULTS AND DISCUSSION

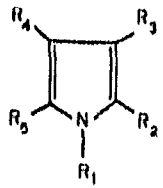
The separation of 14 of the 16 test compounds from zomepirac sodium dihydrate was achieved on a highly efficient microsilica gel column. Table I lists the structures of the test compounds and their respective retention times and minimum detection limits. Compounds 1, 2, 3 and 16 are the only impurities that have been detected in commercial samples of zomepirac sodium dihydrate drug substance. The other compounds are chosen as model compounds to demonstrate the specicity of the method. The glacial acetic acid in the mobile phase modifies the silica surface activity, and zomepirac sodium is eluted in *ca.* 11.2 min. In the absence of glacial acetic acid, zomepirac sodium tails badly and its peak is very broad. Gradient elution is necessary to cover the wide range of elution times of the test compounds.

Fig. 1 shows the separation of a solution of zomepirac sodium dihydrate containing 1.0% of compounds 13 and 15 and 0.4% each of compounds 2, 3, 6, 7, 8 and 9 (Table I). Fig. 2 shows the separation of 0.3% of compounds 1, 5 and 11, and 0.5% of compound 4 from zomepirac sodium. Fig. 3 shows the separation of 0.3% of compounds 12 and 14 from zomepirac sodium.

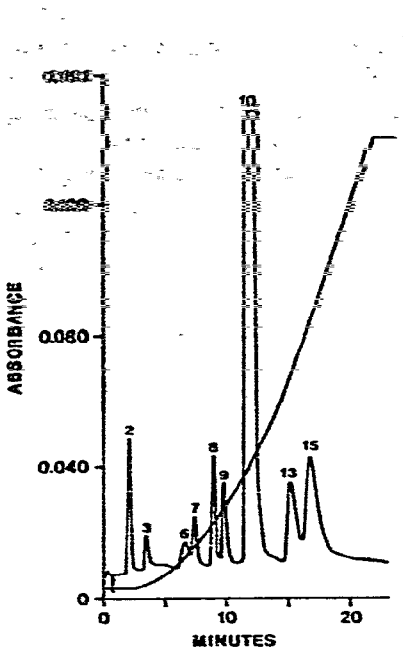
A complete analysis on a silica gel column takes 25 min. For a 100-μg injection of zomepirac sodium dihydrate solution, at least a 0.1% level of compounds 13 and 15, and levels lower than 0.1% of the other compounds, can be detected. To lower further the detection levels of the impurity compounds, a 200-μg sample can be injected. However, a significant tailing effect occurs which overlaps with compounds

TABLE I

HPLC RETENTION TIMES AND MINIMUM DETECTION LIMITS OF THE TEST COMPOUNDS ON SILICA GEL COLUMN



Compound	R_1	R_2	R_3	R_4	R_5	*Retention time (min)*	*Detection limit (μg)*
1	4-Chlorobenzoic Acid	–	–	–	–	1.39	0.02
2	CH_3	CH_3	H	CH_3	$4\text{-}ClC_6H_4CO$	1.61	0.01
3	CH_3	$CH_2COOC_2H_5$	H	CH_3	$4\text{-}ClC_6H_4CO$	2.80	0.04
4	CH_3	$CH_2COOC_2H_5$	$CO_2C_2H_5$	CH_3	H	2.88	0.04
5	CH_3	$CH_2COOC_2H_5$	$CO_2C_2H_5$	CH_3	$4\text{-}ClC_6H_4CO$	2.90	0.02
6	CH_3	CH_2COOCH_3	H	H	$4\text{-}ClC_6H_4CO$	6.49	0.04
7	CH_3	$CH_2COOC_2H_5$	COOH	CH_3	H	6.93	0.04
8	CH_3	$CH_2COOC_2H_5$	COOH	CH_3	$4\text{-}ClC_6H_4CO$	8.44	0.03
9	CH_3	$CH(CH_3)COOH$	H	CH_3	$4\text{-}ClC_6H_4CO$	9.43	0.02
10 (zomepirac)	CH_3	CH_2COOH	H	CH_3	$4\text{-}ClC_6H_4CO$	11.25	–
11	H	CH_2COOH	H	CH_3	$4\text{-}ClC_6H_4CO$	12.37	0.02
12	CH_3	CH_2COOH	$4\text{-}ClC_6H_4CO$	H	CH_3	14.14	0.05
13	CH	CH_2COOH	COOH	CH_3	H	14.65	0.10
14	CH_3	CH_2COOH	H	$4\text{-}ClC_6H_4CO$	CH_3	15.97	0.05
15	CH_3	CH_2COOH	COOH	CH_3	$4\text{-}ClC_6H_4CO$	16.37	0.10



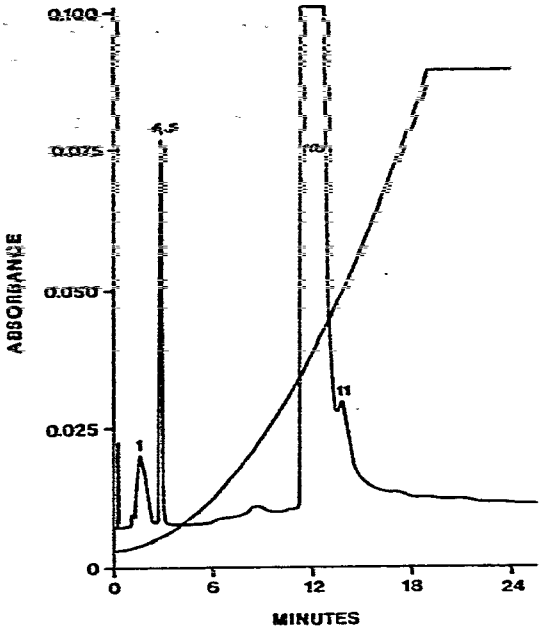
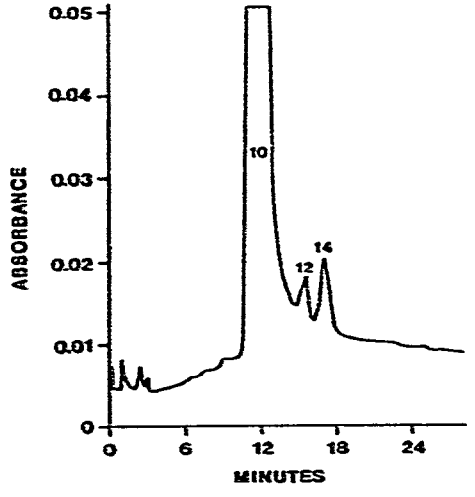


Fig. 1. Separation of zomepirac sodium dihydrate from eight test compounds on a micro silica gel column. See Table I for structures.

Fig. 2. Separation of zomepirac sodium dihydrate from four test compounds on a micro silica gel column. See Table I for structures.



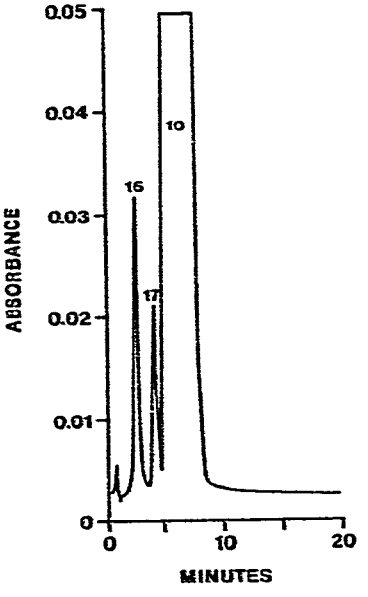


Fig. 3. Separation of zomepirac sodium dihydrate from two test compounds on a micro silica gel column. See Table I for structures. The gradient profile was not recorded.

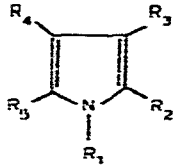
Fig. 4. Separation of zomepirac sodium dihydrate from two test compounds on a reversed-phase column. See Table II for structures.

11–15. Normally, a 100-μg sample is injected, which for all practical purposes exceeds the requirements of a purity method for drug analysis.

The reversed-phase chromatographic method was developed to separate compounds 16 and 17 from zomepirac (see Fig. 4). Compound 17 was not resolved from zomepirac on the silica gel column but it was well separated from zomepirac on the reversed-phase column. Compound 16 did not elute from the silica gel column but it eluted ahead of and was separated from zomepirac on the reversed-phase column. Table II gives the structures of the test compounds, their respective retention times and minimum detection limits. A 100–200 μg sample can be injected onto the reversed-phase column. Though the column was overloaded with respect to zomepirac sodium, the test compounds were well resolved with no appreciable loss of efficiency. The relative standard deviation of the retention time of compound 16 was 2.2% ($n = 5$), and of its peak area was 1.4% ($n = 5$).

TABLE II

HPLC RETENTION TIMES AND MINIMUM DETECTION LIMITS OF THE TEST COMPOUNDS ON THE REVERSED-PHASE COLUMN



Compound	R_1	R_2	R_3	R_4	R_5	*Retention time (min)*	*Detection limit (μg)*
16	CH_3	$CH(OH)COOH$	H	CH_3	4-ClC_6H_4CO	2.49	0.01
17	CH_3	CH_2COOH	H	H	4-ClC_6H_4CO	3.70	0.01
10 (zomepirac)	CH_3	CH_2COOH	H	CH_3	4-ClC_6H_4CO	4.64	—

In the determination of detection limits as listed in Tables I and II, a detector sensitivity of 0.10 a.u.f.s. was used. It is possible to increase the sensitivity to 0.01, if the need arises, to enhance the detection limits.

The retention times of the test compounds are reproducible (Table III). For five repetitive runs, the relative standard deviation was 0.2–2.5%, except compound 2 which was 6.2%. Compound 2 elutes from the column at 1.6 min. Any slight variation of column characteristics will significantly affect the earliest peaks.

The calibration curves of the test compounds show a linear relationship between peak area and amount of sample injected onto a column. A narrow range of amount of test compound was selected (0–1.0 μg) to obtain the calibration curves because it was within this range that an impurity would be present.

The recovery study of representative test compounds on a micro silica gel column (see Table IV) shows that the method accurately determines the amount of each impurity present in a known test mixture. These representative test compounds were selected at random. The purity of the sample was determined by subtracting from 100% each impurity determined.

The columns were stable for several months in routine analytical use. It was

TABLE III

REPRODUCIBILITY OF RETENTION TIMES (min) ON A MICRO SILICA GEL COLUMN

Runs	*Compound*									
	2	*3*	*7*	*8*	*9*	*10*	*12*	*13*	*14*	*15*
1	1.45	2.68	6.90	8.18	9.44	11.25	14.13	14.58	16.01	16.37
2	1.66	2.83	6.93	8.45	9.44	11.24	14.18	14.60	16.04	16.36
3	1.69	2.86	6.96	8.46	9.45	11.29	14.24	14.71	15.95	16.32
4	1.66	2.83	6.94	8.45	9.41	11.29	14.04	14.68	15.94	16.42
5	1.60	2.80	6.90	8.44	9.39	11.23	14.10	14.68	15.93	16.36
Average ($\bar{X}$)	1.61	2.80	6.93	8.40	9.43	11.26	14.14	14.65	15.97	16.37
Standard deviation (σ)	0.10	0.07	0.03	0.12	0.03	0.03	0.08	0.06	0.05	0.04
Relative standard deviation $\left(\frac{\sigma}{\bar{X}} \times 100\%\right)$	6.2%	2.5%	0.4%	1.4%	0.3%	0.3%	0.6%	0.4%	0.3%	0.2%

TABLE IV

RECOVERY STUDY OF REPRESENTATIVE TEST COMPOUNDS ON A MICRO SILICA GEL COLUMN

A 100-μg sample of zomepirac sodium dihydrate containing known amounts of the test compounds was injected onto the HPLC column.

	Compound					
	2	*7*	*8*	*9*	*12*	*10*
Known concn. (%)	0.37	0.32	0.28	0.31	0.41	98.31
Recovered concn. (%)	0.37	0.32	0.26	0.29	0.42	98.34

observed that propan-2-ol dissolved some of the silica gel which caused a void space to appear at the top of the column. When this happened, a small amount of silica gel was added to the top of the column to compensate for this loss. This phenomenon was also observed in another laboratory[6], which reported a way of eliminating this problem by saturating the mobile phase with silica.

In conclusion, the HPLC method has been used to determine the purity of zomepirac sodium dihydrate and follow its stability for up to four years. Hundreds of samples have been tested and satisfactory results were obtained.

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