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Note

Determination of thenoyl peroxide by high-performance liquid chromatography

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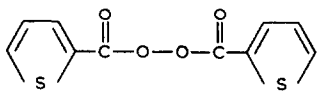
Thenoyl peroxide is a structural analogue of benzoyl peroxide, well known for its desquamative, keratolytic and antibacterial properties[1]. We have developed a



method which has the necessary sensitivity for simultaneous quantitation of ng levels of thenoyl peroxide and thenoic acid in drugs, using reversed-phase high-performance liquid chromatography (HPLC). It is similar to that previously published by us for the quantitation of benzoyl peroxide in dermics[2].

EXPERIMENTAL

Materials

Acetonitrile was "for HPLC" grade (Fluka, art. No. 692) and was used without further purification. Water was obtained through a Millipore installation and its resistivity was at least 18 MΩ. It was filtered through a $0.22 \cdot 10^{-6}$-m Sartorius filter. Thenoic acid was obtained from Syntha (Suresnes, France) and thenoyl peroxide from Roussel UCLAF (Paris, France).

Chromatographic conditions

The analysis was performed on a high-performance liquid chromatograph (Perkin-Elmer Sigma 1) equipped with a UV absorbance spectrophotometer (Perkin-Elmer LC 85) connected to an automatic programmable integrator (Perkin-Elmer Sigma 10). The injection was made through a septum-less injector equipped with a 10-μl sample loop (Rheodyne Model 71-25). A 300 × 3.9 mm μBondapak C_{18} column (Waters) was used at a flow-rate of 1.2 ml/min, and pressure of 1400 p.s.i. Detection was monitored at 232 nm and the sensitivity was 0.1 absorbance units full scale.

Under these conditions, retention times were 2.12 min for thenoic acid and 4.16 min for thenoyl peroxide (Fig. 1).

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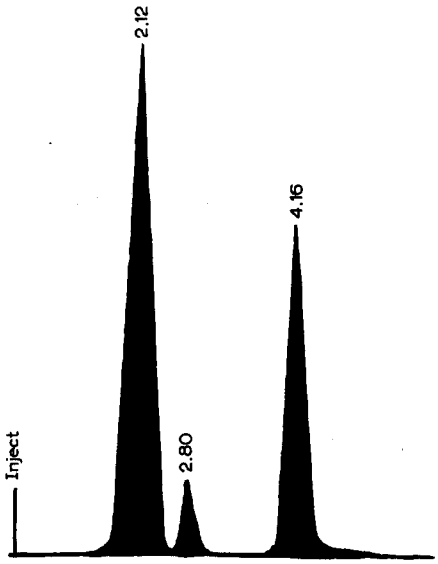

Fig. 1. Typical chromatogram obtained by injection of a sample solution. For chromatographic conditions, see Experimental.

Thenoyl peroxide standard

A 100-mg amount of thenoyl peroxide was weighed accurately into a 10-ml volumetric flask. Acetonitrile was added and the flask was shaken until the compound dissolved. The sample was then made up to volume with acetonitrile. Dilutions were made with this stock solution to obtain solutions containing 2, 1, 0.5, 0.2, 0.1 and 0.02 mg/ml.

Preparation of eluent

A mixture of 750 ml acetonitrile and 250 ml of 18-MΩ water was stirred magnetically for 15 min. The whole solution was then filtered on a 0.22-μm filter (Sartorius, 11607) and then degassed with helium for 10 min.

Sample preparation

A 250-mg amount of gel (or lotion) was weighed accurately into a 25-ml volumetric flask, 15 ml of acetonitrile were added and the flask was shaken until the compound dissolved. The sample was made up to volume with acetonitrile. This solution was then filtered through a 0.45-μm inert filter into a 50-ml volumetric flask. The original flask and filter were washed with 3 × 5 ml of acetonitrile. The sample was finally made up to volume with water.

Method of quantitation

The peak area for thenoyl peroxide was measured by computer (Perkin-Elmer, Sigma 10). A calibration curve for known concentrations was prepared by plotting the peak area for each concentration. Unknown concentrations in specimens were calculated by the computer from standard areas of calibration solutions having sim-

ilar concentrations to that expected in the samples. All injections were made on the same day in the sequence: three samples, one standard and so on.

RESULTS AND DISCUSSION

Linearity

Thenoyl peroxide showed a linear response from 0.4 to 40 μg injected. The linear correlation coefficient was found to be 0.99967 and the intercept was less than 1% of the response for the normal concentration injected (Table I).

TABLE I

LINEARITY OF THE HPLC METHOD FOR THENOYL PEROXIDE

Results based on five replicate injections, given as the mean ± S.D. Slope = 0.989. Intercept = 14.7. Correlation coefficient determined by linear regression analysis: 0.99967.

Amount injected (μg)	*Peak area (integrator units)*
0.4	1747 ± 130.4
2	8542 ± 294.4
4	20,391 ± 251
10	44,528 ± 608
20	85,484 ± 1152
40	160,184 ± 2910

Chromatographic precision

The chromatographic precision was determined by making eight injections from a single freshly prepared solution of thenoyl peroxide in water on each of three days. The precision was calculated each day as the relative standard deviation, and the chromatographic precision was taken to be the average of the three relative standard deviations, *i.e.*, 1% (Table II).

TABLE II

DAY-TO-DAY REPRODUCIBILITY OF INJECTION OF REPLICATE (200 μg/ml) SAMPLES OF THENOYL PEROXIDE

Results based on eight replicate injections, given as the mean ± S.D. Mean R.S.D.: 1%.

Day	*Peak area (integrator units)*	*Relative standard deviation (%)*
1	37,801 ± 663	1.7
2	37,796 ± 344	0.9
3	37,836 ± 158	0.4

TABLE III

DETERMINATION OF THENOYL PEROXIDE: ACCURACY AND ASSAY PRECISION

	Sample (250 mg)		
	1	*2*	*3*
Peak area detected at 232 nm (integrator units)	51,505 54,325 54,164 52,983	52,272 53,393 53,093 53,008	52,968 53,569 53,849 53,340
Mean area	53,244	52,941	53,431
Recovery (%)	99.8	99	99.9

Accuracy and assay precision

An estimate of the procedural accuracy was obtained by assaying three replicate extractions *versus* a standard (Table III). The relative standard deviation of the recoveries was used as an estimate of assay precision (0.46%).

Detection limit

The detection limit was estimated by plotting and running a "blank solution" under the same conditions as for the sample. The detector was at its maximum sensitivity (0.01 absorbance units full scale) and the reporting integrator was used in the

TABLE IV

THENOYL PEROXIDES GELS AND LOTIONS: RESULTS OF STABILITY STUDIES

Gel: 10 g thenoyl peroxide, 6 g Brij 35, 5 g acetone, 2 g Carbopol, purified water sufficient quantity for 100 ml. *Lotion*: 10 g thenoyl peroxide, 2 g cetyl alcohol, 0.5 g sodium laurylsulphate, 1 g propylene glycol, purified water sufficient quantity for 100 ml.

	Gel 1	*Gel 2*	*Gel 3*	*Lotion 1*	*Lotion 2*	*Lotion 3*
Storage* temperature (°C)	4	20	37	4	20	37
Peak area detected**	50,135	64,763	64,134	49,516	55,429	44,800
Standard deviation	351	647	320	495	277	448
Sample weight (mg)	228	292	470	239	257	250
Concentration*** (g/100 ml)	8.9	8.9	5.52	8.37	8.71	7.25

* Samples were stored in darkness.
** Mean value from eight determinations.
*** Calculated value: 9 g/100 ml.

faster acquisition mode. The baseline noise was then integrated and averaged. It was found to be 0.05 integrator units.

The detection limit was considered to be the amount of thenoyl peroxide and thenoic acid which will give a peak area of three times the mean baseline noise. This amount was 1 ng for thenoyl peroxide (area of 0.15) and 2 ng for thenoic acid (area of 0.18).

Application to dosage forms

The dosage forms containing thenoyl peroxide formulated in this laboratory were determined using this method. The data in Table IV show the results obtained.

REFERENCES

1 *French Pat.*, 2,420,972 (1980).
2 C. Ehinger and G. Andermann, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 3 (1980) 143–144.