Journal of Chromatography, 549 (1991) 217–220
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 317

Separation and simultaneous high-performance liquid chromatographic determination of benzocaine and benzyl benzoate in a pharmaceutical preparation

BÁRBARA GIGANTE, ANA M. V. BARROS, ADRIANO TEIXEIRA and M. J. MARCELO-CURTO*

Laboratório Nacional de Engenharia e Tecnologia Industrial, Departamento de Tecnologia de Indústrias Químicas, Estrada das Palmeiras, 2745 Queluz (Portugal)

(First received January 21st, 1991; revised manuscript received March 8th, 1991)

ABSTRACT

A simple reversed-phase high-performance liquid chromatographic method suitable for the simultaneous determination of benzocaine and benzyl benzoate in dermatological preparations is described. An internal standard method was employed, using a C_{18} "bonded phase" silica column and a mobile phase consisting of acetonitrile–water (40:60, v/v), with absorption of the column effluent monitored at 254 nm. No sources of interference were observed. The simultaneous determination of both compounds by the method described is rapid and accurate.

INTRODUCTION

Benzyl benzoate, a potent acaricide, and benzocaine (ethyl *p*-aminobenzoate), a local anesthetic, are formulated together in dermatological preparations used against scabies and pediculosis. No methods have been reported for the quantification of benzyl benzoate, and although several methods have been described for the quantification of benzocaine [1–3] none is suitable for the simultaneous determination of both compounds. In this paper, we describe a high-performance liquid chromatographic (HPLC) procedure in which both compounds can be quantified simultaneously under the same chromatographic conditions.

EXPERIMENTAL

Chemicals

HPLC-grade methanol and acetonitrile were used (Merck, Darmstadt, Germany). Water was purified with a Millipore filtration unit (deionized, $< 10 \mu\Omega$).

Reference standards and standard solutions

Benzocaine, benzyl benzoate and benzophenone (internal standard) were analytical-grade reagents (Merck); Neo-Escabenzil and other dosage forms were kindly donated by Companhia Portuguesa Higiene (Lisbon, Portugal).

0021-9673/91/$03.50

An internal standard stock solution of benzophenone in methanol at a concentration of 1.5 mg/ml was used. The reference stock solution was prepared by accurately weighing about 0.02 g of benzocaine and 0.15 g of benzyl benzoate and dissolving with methanol in a 10-ml volumetric flask. The reference solution was prepared by measuring 1.0 ml of the reference stock solution and 1 ml of internal standard stock solution into a 10-ml volumetric flask and diluting with methanol.

Standard solutions for calibration graphs. Appropriate volumes were measured out of the reference solution, mixed with the internal standard and diluted to 10 ml with methanol. The calibration was carried over a concentration range of 0.01–0.5 mg/ml for benzocaine and 0.07–0.30 mg/ml for benzyl benzoate.

Apparatus

A Spectra-Physics (San Jose, CA, USA) high-performance liquid chromatograph equipped with a Rheodyne 10-μl loop injector valve, a double stage pump Ioschrom LC and a variable-wavelength UV detector Spectra-Chrom 100 were used. The wavelength was set at 254 nm. The chromatographic peaks were recorded with a Spectra-Physics SP 4270 computing integrator connected to the spectrophotometer, with an operating voltage of 10 mV and chart speed of 10 mm/min. A 22 × 0.46 cm I.D. stainless-steel column containing C_{18} "bonded phase" silica, 5 μm, with a 3 cm guard column (Brownlee Labs., Santa Clara, CA, USA) and a 10-μl injector loop were employed. The injection volume was 50 μl. A flow-rate of 2 ml/min eluted benzocaine and benzyl benzoate in 1.8 and 4.5 min, respectively. All analyses were performed at room temperature.

Mobile phase and stability of chromatographic system

The mobile phase consisted of acetonitrile–water (60:40, v/v), filtered through a 0.2-μm PTFE membrane and degassed first in an ultrasonic bath and then by helium flow. The column was equilibrated with mobile phase at a flow-rate of 2 ml/min.

Sample preparation

The sample solution was prepared by accurately weighing about 0.05 g of the sample, mixing with 5 ml of internal standard stock solution and diluting with methanol in a 50-ml volumetric flask. The injection solution was prepared by accurately measuring 1 ml of the sample solution and diluting with methanol in a 10-ml volumetric flask. This solution was filtered through a 0.45-μm PTFE membrane prior to HPLC analysis.

RESULTS AND DISCUSSION

Although other HPLC methods have been described for the quantification of benzocaine [1–3], trials to separate benzocaine and benzyl benzoate simultaneously by the methods reported were unsuccessful.

Fig. 1 depicts a chromatogram of the commercial formulation Neo-Escabenzil, typically containing 2% benzocaine and 15% benzyl benzoate, showing the HPLC method proposed here to be suitable for the simultaneous determination of both compounds. Benzocaine and benzyl benzoate were eluted in 1.8 and 4.5 min, respectively, from standard and sample solutions.

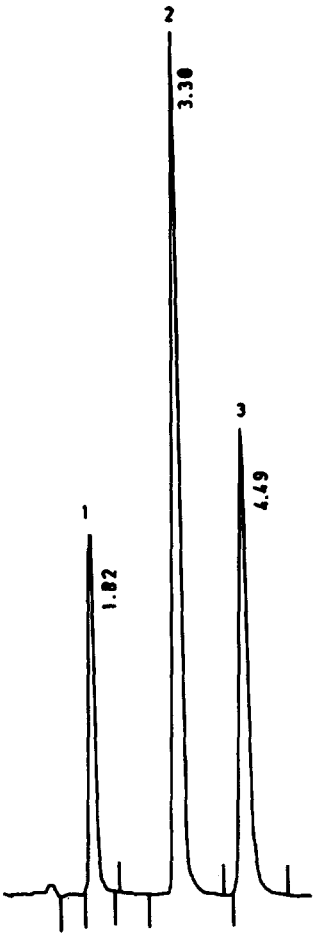

Fig. 1. Chromatogram of a sample of Neo-Escabenzil at 254 nm. Peaks: 1 = benzocaine (0.022 mg/ml; retention time 1.82 min); 2 = benzophenone (internal standard, 1.5 mg/ml; retention time 3.30 min); 3 = benzyl benzoate (0.157 mg/ml; retention time 4.49 min).

Adequate separation of benzocaine and benzyl benzoate was achieved with a mobile phase consisting of acetonitrile–water (60:40, v/v), pH adjustment being unnecessary to reach optimum chromatographic conditions. The column was equilibrated with mobile phase at a flow-rate of 2 ml/min. The relative standard deviation (R.S.D.) of five replicate injections of a standard was not more than 1.8%.

The wavelength of 254 nm was selected for detection according to the absorbance maxima of benzocaine, benzyl benzoate and their concentrations in the sample solution.

An internal standard method was employed, with benzophenone as the internal standard. No interference from additives or diluents was detected upon application of the analytical procedure to commercial formulations.

Linearity of detector response to variations in concentration was determined

TABLE I

CALIBRATION DATA FOR ANALYSIS

Compound	Concentration range (mg/ml)	Correlation coeffient ($n=5$) ± R.S.D. (%)	Slope	Intercept	Retention time (min)
Benzocaine	0.01–0.50	0.9991 ± 0.038	0.0094	0.0073	1.80
Benzyl benzoate	0.07–0.30	0.9994 ± 0.180	0.0023	0.0002	4.50

over a range of 0.01–0.50 mg/ml for benzocaine with a correlation coefficient of 0.9991 ($n=5$) and 0.07–0.30 mg/ml for benzyl benzoate with a correlation coefficient of 0.9994 ($n=5$). Calibration graphs were constructed of peak area *versus* concentration; the slope and intercept obtained by linear regression analysis are listed in Table I and the results show the low R.S.D. values between analysis. The reproducibility of the method was tested with repeated analysis of samples ($n=11$) corresponding to the average weight of the samples.

The accuracy of the method was determined by calculating the recovery of known amounts of the authentic sample solution added to the average weight of the assay samples. Recoveries were in the range 100–103% for benzocaine and 97–100% for benzyl benzoate.

This method is simple, specific, accurate and reproducible and therefore can be applied to various commercial pharmaceutical preparations.

REFERENCES

1 I. Jane, A. McKinnon and R. J. Flanagan, *J. Chromatogr.*, 323 (1985) 191.
2 R. Gill, R. W. Abbott and A. C. Moffat, *J. Chromatogr.*, 301 (1984) 155.
3 M. Bhuze, S. Fregert and B. Gruvberger, *Photodermathology*, 1 (1984) 277.