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Note

Determination of bufexamac in cream and ointment by high-performance liquid chromatography

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Bufexamac (4-butoxy-N-hydroxybenzeneacetamide) is a drug possessing analgesic, anti-inflammatory and antipyretic effects[1–4]. It is used extensively for the treatment of contact dermatitis and acute eczema.

Some analytical procedures have been reported for the quantitation of bufexamac. In 1966, Lambelin *et al.*[5] described a colorimetric method for the determination of bufexamac in serum. This method lacks specificity because it detects not only bufexamac but also its hydroxy derivatives. Roncucci *et al.*[6] and Dell *et al.*[7] described a gas–liquid chromatographic method for the determination of bufexamac in plasma and urine. However, these methods are very tedious and time-consuming because they require a derivatization step before analysis.

In this paper, a rapid, specific and sensitive high-performance liquid chromatographic (HPLC) method for the quantitation of bufexamac in cream and ointment is described.

EXPERIMENTAL

Apparatus

The HPLC unit consisted of a JASCO pump Model BIP-I (Japan Spectroscopic Co., Tokyo, Japan), a JASCO Model UVIDEC-100 V variable-wavelength UV detector set at 280 nm and an injector Model 7125 fitted with a 20-μl loop (Rheodyne, Cotati, CA, U.S.A.). The HPLC system was linked to a Chromatopac C-R3A digital integrator (Shimadzu, Kyoto, Japan).

Chromatographic conditions

The HPLC column used was a 30 cm × 4 mm I.D. μBondapak C_{18} (Waters), particle size 8–10 μm. The mobile phase was 60% (v/v) methanol in water. The pH of the mobile phase was adjusted to 3.0 by adding phosphoric acid. It was then filtered through a Millipore membrane filter (0.45 μm; Millipore, Bedford, MA, U.S.A.) and degassed before use. The sample was chromatographed using a flow-rate of 1.0 ml/min at ambient temperature. Samples of 1 μl were injected.

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Reagents

Bufexamac (Sigma) and *n*-butyl *p*-hydroxybenzoate (Tokyo Kasei) were used as received. All other solvents used were of reagent grade (Wako). Distilled water was used when preparing the mobile phase.

Calibration curve

A stock standard solution of bufexamac was prepared in methanol (10.0 mg/ml). A solution of *n*-butyl *p*-hydroxybenzoate in methanol (approximately 10 mg/ml) was used as an internal standard solution. Aliquots of the standard stock solution, equivalent to 20, 40, 60, 80 and 100 mg, were pipetted into a 100-ml volumetric flask and aliquots of the internal standard solution, equivalent to 10 mg, were added. The resulting solutions were diluted to volume in methanol to obtain the working standard solutions. These solutions were used for calibration purposes. The calibration curve was constituted from the values of the ratio of the peak height of bufexamac to that of the internal standard.

Analysis of bufexamac samples

Approximately 1 g of sample (*ca.* 50 mg of bufexamac) was accurately weighed into a 200-ml separating funnel, 100 ml of hexane and 30 ml of methanol–water (8:2) were added and shaken well for 10 min on a shaker. The layers were allowed to separate, and the lower layer was drawn off into a 100-ml volumetric flask. The extraction was repeated with 30 ml of methanolic solution, and the methanol extracts were pooled; 1 ml of the internal standard was added, and the mixture was diluted to volume in methanol. A 1-μl aliquot of this solution was injected into the chromatograph; the amount of bufexamac present was calculated using the calibration curve.

RESULTS AND DISCUSSION

The elution characteristics of bufexamac on three reversed-phase ODS columns from different manufacturers were compared using an aqueous methanol (pH 3.0) mobile phase. The separation parameters, *i.e.*, the number of theoretical plates,

TABLE I

CALCULATED SEPARATION PARAMETERS FOR THE THREE ODS COLUMNS

Columns: A = LiChrosorb RP-18 (7 μm, 250 × 4.0 mm I.D.); B = Zorbax C_{18} (5–6 μm, 150 mm × 4.6 mm I.D.); C = μBondapak C_{18} (8–10 μm, 300 mm × 3.9 mm I.D.). Mobile phase: methanol–water (pH 3) (6:4, v/v); flow-rate 1 ml/min. Detection: UV, 280 nm. N = Number of theoretical plates per 25 cm, calculated for bufexamac; I.S. = *n*-butyl *p*-hydroxybenzoate; S = asymmetry factor, calculated as defined by Kirkland[8].

Column	N	k'		α *for bufexamac/I.S.*	S
		Bufexamac	*I.S.*		
A	136	2.8	5.5	2.34	4.13
B	152	2.9	4.4	1.68	3.37
C	4466	2.6	3.5	1.65	1.08

TABLE II

DETERMINATION OF BUFEXAMAC IN COMMERCIAL SAMPLES BY HPLC

Sample	*Formulation*	*Found**	
		mg/g	*% of declared*
1	Cream	48.6	97.2
2	Cream	51.6	103.2
3	Cream	50.0	100.0
4	Cream	48.1	96.2
5	Cream	48.7	97.4
6	Ointment	48.9	97.8
7	Ointment	49.2	98.4
8	Ointment	45.6	91.2

* Based upon three determinations for each sample. Amount declared was 50 mg/g in each case.

N, selectivity, α, peak asymmetry, S, and capacity factor, k', were calculated and are listed in Table I. It was found that columns from different manufacturers had significantly different behaviours with respect to separation parameters for bufexamac. Such variations in separation parameters among ODS columns from different manufacturers is strong evidence that column characteristics, such as the percentage of hydrocarbon loading, type of organosilanes used for the preparation of bonded hydrocarbon and the number of unsilanized hydroxy sites, can be as important as the type of the bonded hydrocarbon in determining separation parameters.

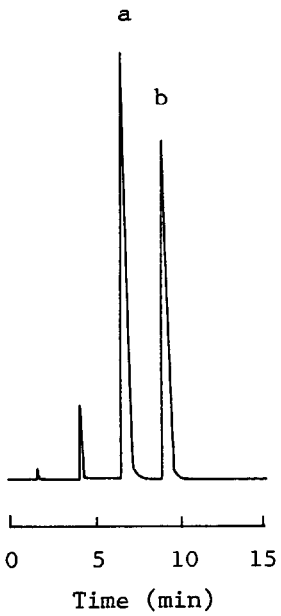

Fig. 1. Typical HPLC chromatogram of bufexamac in an ointment sample. HPLC conditions: μBondapak C_{18} column; mobile phase, 60% (v/v) methanol in water (pH 3); ambient temperature; flow-rate 1 ml/min and UV detection at 280 nm. Peaks: a = bufexamac; b = *n*-butyl *p*-hydroxybenzoate (internal standard).

The μBondapak C_{18} column was the most suitable for this analysis. The other columns did not demonstrate the efficiency required to separate bufexamac.

To optimize the system, the influences of the methanol concentration and of the pH on the separation were investigated; 60% (v/v) methanol in water and pH 3.0 were chosen as the optimum eluent parameters.

This assay method for bufexamac on μBondapak C_{18} has been developed using the internal standard technique. *n*-Butyl *p*-hydroxybenzoate is an ideal internal standard. It allows the HPLC assay to be completed in about 9 min. Both bufexamac and the internal standard have UV absorption maxima around 280 nm. The relationship between the peak-height ratio relative to *n*-butyl *p*-hydroxybenzoate and the amount of bufexamac was linear over the range selected, 0.2–1.0 mg/ml, with a correlation coefficient of 0.999.

The accuracy of the procedure was determined by spiking a placebo formulation (cream and ointment) with known concentrations of standard. Recoveries of bufexamac averaged 99.7% ($n = 6$) with a relative standard deviation (R.S.D.) of 1.02% for bufexamac levels of 50 mg/g.

Table II reports the results obtained in the analysis of commercial samples of bufexamac and Fig. 1 shows a typical chromatogram obtained from a commercial sample.

These results indicate that this method is suitable for the determination of bufexamac in cream and ointment.

REFERENCES

1 N. P. Buu-Hoi, G. Lambelin, C. Lepoivre, C. Gillet, M. Gautier and J. Thriaux, *C.R.*, 261 (1965) 2259.
2 N. P. Buu-Hoi, G. Lambelin, G. Mees, C. Gillet and J. Thiriaux, *C.R.*, 261 (1965) 4907.
3 G. Lanbelin, N. P. Buu-Hoi, G. Mees, J. Thiriaux and M. Huriaux, *Med. Pharmacol. Exp.*, 15 (1966) 307.
4 N. P. Buu-Hoi, G. Lanbelin, C. Gillet, C. Lepoivre, J. Thiriaux and G. Mees, *Nature (London)*, 211 (1966) 752.
5 G. Lambelin, R. Ronucci, M.-J. Simon, N. P. Buu-Hoi and J. Thiriaux, *Biochem. Pharmacol.*, 15 (1966) 1563.
6 R. Roncucci, M.-J. Simon and G. Lambelin, *J. Chromatogr.*, 57 (1971) 410.
7 D. Dell, D. R. Boreham and B. K. Martin, *J. Pharm. Sci.*, 60 (1971) 1368.
8 J. J. Kirkland, W. W. Yau, H. J. Stobbosa and C. H. Diika, *J. Chromatogr. Sci.*, 15 (1977) 303.