*Journal of Chromatography*, 249 (1982) 373–378
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

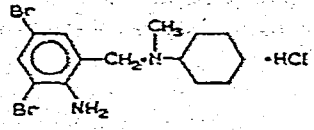
CHROM. 15,175

## Note

# Determination of bromhexine hydrochloride in pharmaceutical preparations by reversed-phase ion-pair high-performance liquid chromatography

J. L. KUMAR*, W. C. MANN and A. ROZANSKI
*International Development Centre, Abbott Laboratories, Queenborough, Kent ME11 5 EL (Great Britain)*
(Received July 8th, 1982)

Due to its bronchial mucolytic action, bromhexine hydrochloride [N-(2-amino-3,5-dibromobenzyl)-N-cyclohexyl-methylamine hydrochloride] has been presented alone (Bisolvon®; Boehringer, Ingelheim, G.F.R.), and has also been incorporated in pharmaceutical preparation with other drugs and is presented commercially as tablets and granules [Pantobron®, Erybron®, Synergomycin®, Pantobron Forte® (Abbott Labs.); Bisolvonat®, Bisolvomycin® (Boehringer)]. An accurate, precise and specific analytical method for the rapid determination of bromhexine hydrochloride in these and experimental formulations was required.




$C_{14}H_{20}Br_2N_2 \cdot HCl$ 412.6

Bromhexine hydrochloride

A gas chromatographic (GC) method has been described[1] for the assay of bromhexine hydrochloride in pharmaceutical preparations, and another GC method[2,3] for its determination in biological fluids. In the latter case derivatisation with trifluoroacetic anhydride is necessary. These methods were not applicable for the determination of bromhexine in pharmaceutical formulations containing high concentrations of antibiotics such as erythromycin ethylsuccinate[4].

We have in current use in our laboratories a method for the analysis of bromhexine hydrochloride which involves thin-layer chromatographic separation of bromhexine hydrochloride from other drugs followed by spectrophotometric determination. This method is very laborious and time-consuming, and an improved method was desirable.

This paper reports a new, sensitive and specific high-performance liquid chromatographic (HPLC) system for the determination of bromhexine hydrochloride. The method requires only dissolution of the sample and dilution prior to chromatography to provide complete separation and quantification of bromhexine hydrochlo-

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ride within 15 min. This method has been used to assay a number of formulations and to monitor stability programmes.

## EXPERIMENTAL

### *Chemicals and reagents*

Reagents and solvents were obtained from BDH (Poole, Great Britain) and were AnalaR grade, except for sodium lauryl sulphate (BDH Biochemical). The bromhexine hydrochloride used was an approved batch of raw material. All chemicals and reagents were used as received.

*0.1 M Ethanolic hydrochloric acid.* Concentrated hydrochloric acid (9 ml) was diluted to 1000 ml with ethanol. It is essential that absolute ethanol is used, and that the prepared reagent contains less than 1 % of water.

*Mobile phase.* 0.005 *M* Sodium lauryl sulphate in methanol–water–glacial acetic acid (75:25:0.1). This was prepared freshly each day and was filtered and deaerated before use.

*Bromhexine hydrochloride stock solution.* Bromhexine hydrochloride (*ca.* 30 mg), accurately weighed, was dissolved in and diluted to 50 ml with 0.1 *M* ethanolic hydrochloric acid.

### *Apparatus*

A Waters Model 6000A solvent delivery system (Waters Assoc., Northwich, Great Britain) was used with a Cecil Instruments CE 2012 ultraviolet monitor (Cecil Instruments, Cambridge, Great Britain) operated at 245 nm with a sensitivity of 0.05 a.u.f.s. Injections were made with a Rheodyne 7120 valve (Rheodyne, U.S.A.) fitted with 20-$\mu$l sample loop. The separation was achieved using Waters Assoc. $\mu$Bondapak $C_{18}$ column.

### *Calibration graph*

Aliquots (2.5, 5, 10, 15 and 20 ml) of the bromhexine hydrochloride stock solution were transferred into separate 100-ml graduated flasks, and the contents were diluted to volume with 0.1 *M* ethanolic hydrochloric acid. The solutions were subjected to chromatography and the peak areas of the resulting chromatograms were measured using an integrator. A calibration graph was prepared by plotting the area of the bromhexine hydrochloride peak *versus* concentration. The standard solutions were chromatographed both at the beginning and at the end of a day's run and the results averaged. A typical chromatogram of the standard is shown in Fig. 1.

### *Assay procedure*

Duplicate portions of finely powdered tablets, formulated granules, or liquid formulation, each containing *ca.* 3.0–3.5 mg of bromhexine hydrochloride, were transferred quantitatively into 50-ml volumetric flasks and the contents were diluted with 40 ml of 0.1 *M* ethanolic hydrochloric acid. The contents of the flasks were shaken vigorously on a mechanical shaker for 30 min, diluted to volume with 0.1 *M* ethanolic hydrochloric acid and mixed well. A 10-ml portion of the liquid was filtered through a 0.7-$\mu$m glass fibre filter (Whatman GF/F). These solutions were subjected to chromatography, and the peak areas of the resulting chromatograms were com-

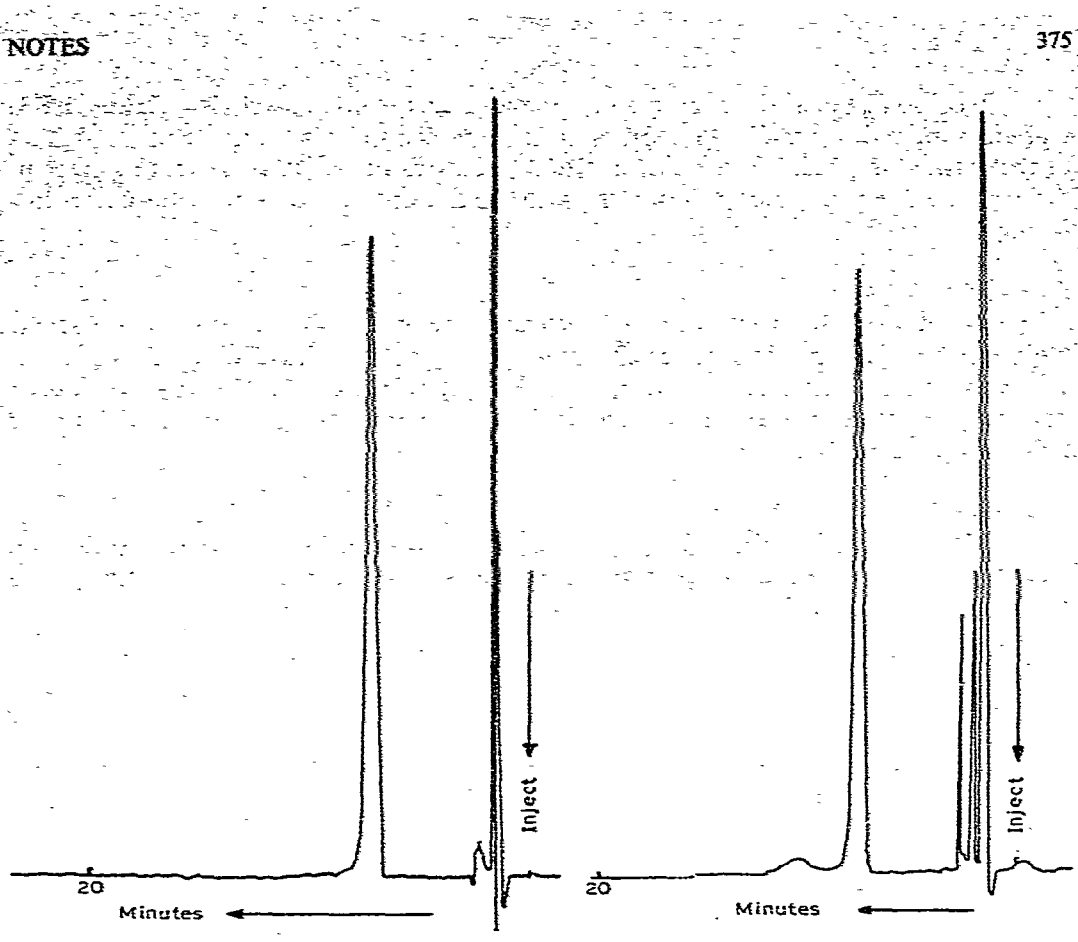


Fig. 1. Chromatogram of bromhexine hydrochloride standard.

Fig. 2. Chromatogram of typical formulation containing bromhexine hydrochloride.

pared with the calibration graph and the concentration of bromhexine hydrochloride in the sample calculated.

A typical chromatogram obtained from the analysis of a formulated granules is shown in Fig. 2.

## RESULTS AND DISCUSSION

*Choice of system*

It is necessary to use an ion-pairing reagent because when the mobile phase consists of water–methanol (25:75) only, bromhexine is eluted as a very low, distorted peak; when water–methanol–acetic acid (25:75:0.1) is used, bromhexine is eluted as a very sharp but non-retained peak.

The use of a lipophilic counter ion (lauryl sulphate) gives a sharp peak and desired retention of bromhexine.

The detector wavelength, 245 nm, was optimised for bromhexine by recording the UV spectrum of bromhexine in 0.1 *M* methanolic hydrochloric acid. There is sufficient absorption at the maximum for reasonable sensitivity.

No interference from excipients or from erythromycin ethylsuccinate (EES) was encountered. A small peak, retention time *ca.* 9 min, was found to be an artifact of the extraction process arising from EES. This extra peak, although eluting close to the bromhexine peak, did not interfere with the quantitative determination of bromhexine.

*Extraction*

Bromhexine hydrochloride was found to be more soluble in 0.1 *M* ethanolic hydrochloric acid and give better chromatography than with other solvents such as water, methanol, ethanol or 0.1 *M* methanolic hydrochloric acid. Water was not used as it also dissolved, for example, various sugars which would be precipitated on the column by the mobile phase.

*Calibration*

The results of a calibration experiment are shown in Fig. 3. No deviation from linearity was observed over the range studied. A straight line with essentially zero intercept was obtained.

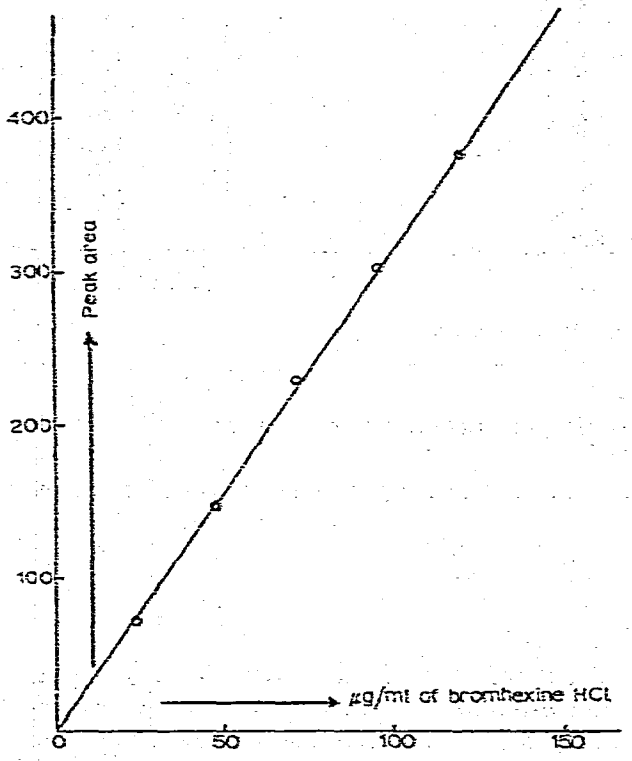


Fig. 3. Calibration of bromhexine hydrochloride peak area *vs.* concentration. Least squares curve fit; $y = -0.6 + 3.141x$, correlation coefficient = 0.9997.

*Reproducibility*

By repeated injection of the same sample solution the relative standard deviation of the chromatographic procedure was determined to be 0.96% ($n = 9$).

TABLE I

REPRODUCIBILITY OF THE HPLC ASSAY BY TWO OPERATORS, OVER 2 DAYS, OF A SAMPLE OF A TYPICAL FORMULATION

S.D. = Standard deviation; R.S.D. = relative standard deviation.

| *Analyst* | *Bromhexine HCl (mg/g)* | | | |
|---|---|---|---|---|
| | *Found* | *Mean* | *S.D.* | *R.S.D. (%)* |
| I | 2.29, 2.26, 2.25, 2.24, 2.21, 2.18 | 2.24 | 0.04 | 1.73 |
| II | 2.25, 2.23, 2.29, 2.22, 2.16, 2.36 | 2.25 | 0.07 | 3.02 |
| Overall (two analysts) | | 2.245 | 0.05 | 2.37 |

The relative standard deviation on six-fold duplicate determination of bromhexine hydrochloride in one formulation was 1.2%.

The relative standard deviation of the twelve-fold replicate determination of bromhexine hydrochloride in another formulation was 2.37%, see Table I. This exercise was done by two operators and spread over 2 days. The small standard deviation demonstrated that bromhexine hydrochloride can be measured with acceptable precision.

*Recovery*

Two samples of a formulated granule were spiked with an additional 50% and 100% bromhexine hydrochloride. The assay results, plotted in Fig. 4, indicated a mean recovery of 98.2% and showed satisfactory linearity of amount added against amount found.

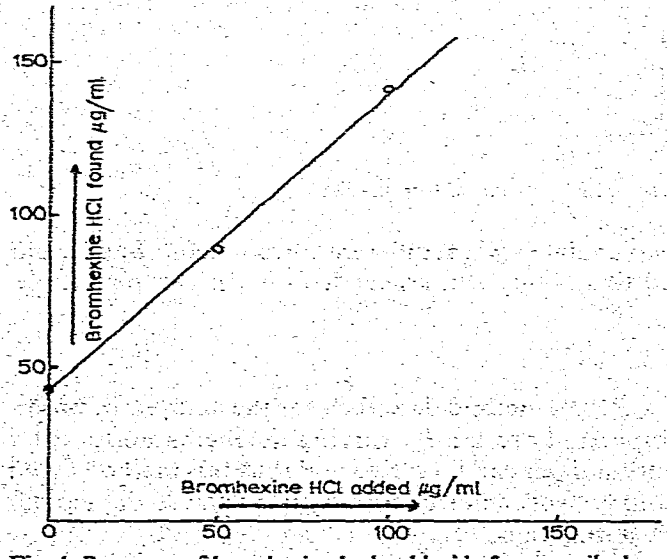


Fig. 4. Recovery of bromhexine hydrochloride from a spiked sample of formulated granules.

*Stability indicating HPLC assay*

Since HPLC involves chromatographic separation, the technique usually provides a stability indicating assay. This is shown for the present assay by a diminished bromhexine peak and a number of additional peaks in a formulation subjected to extreme thermal stress (Fig. 5).

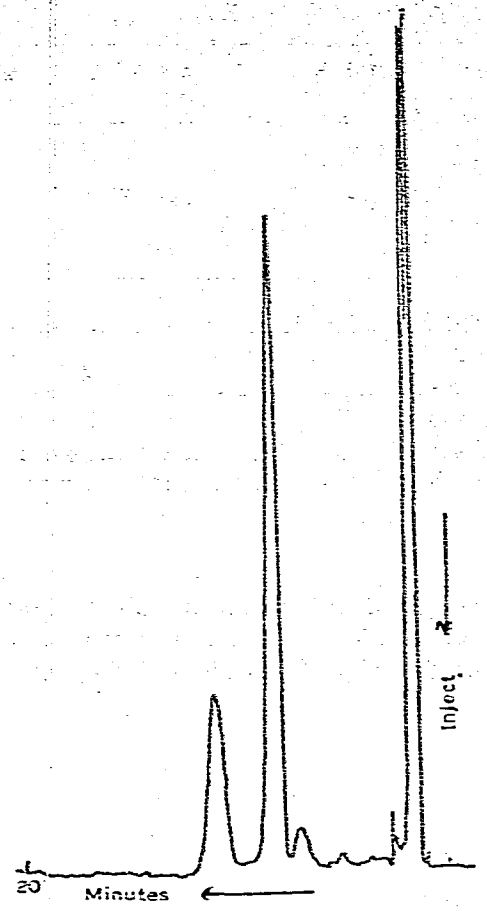


Fig. 5. Chromatogram of thermally stresses sample of typical formulation.

The method has been used to analyse several different formulated products and has also been successfully applied to stability studies carried out on various formulations.

## CONCLUSIONS

The reversed-phase ion-pair HPLC method described for the analysis of bromhexine hydrochloride is simple, rapid and precise. By varying the methanol-to-water ratio in the mobile phase the retention times can be varied and thus this method could easily be adapted to other bromhexine hydrochloride formulations and to the separation of bromhexine from biological materials.

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