Journal of Pharmaceutical & Biomedical Analysis
Vol. 10, No. 2/3, pp. 213–217, 1992
Printed in Great Britain

0731–7085/92 $5.00 + 0.00

Chromatographic analysis of 1,3-bis(dimethylamino)isopropyl 4-chlorophenoxyacetate dihydrochloride, a new CNS stimulant

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Abstract: A reversed-phase liquid chromatographic method is developed for the assay of the new CNS stimulant, 1,3-bis(dimethylamino)isopropyl 4-chlorophenoxyacetate dihydrochloride (BCE-001), and its main contaminant, 4-chlorophenoxyacetic acid (PCPA). In the first place the possible reactions of BCE-001 with mobile phase components are investigated and it is found that BCE-001 is readily hydrolysed in water and undergoes transesterification in methanol or ethanol. The methyl ester is formed rapidly and quantitatively so that BCE-001 can be assayed as methyl 4-chlorophenoxyacetate. PCPA is formed as a result of the hydrolysis of BCE-001 during the sample pretreatment and chromatographic separation and this causes an overestimate of the PCPA impurity in the bulk drug. An effective method is developed to prevent the hydrolysis of BCE-001 during sample pretreatment.

Keywords: *1,3-bis (dimethylamino)isopropyl 4-chlorophenoxyacetate assay; analyte hydrolysis; pre-column methylation; reversed-phase LC.*

Introduction

1,3-bis(dimethylamino)isopropyl 4-chlorophenoxyacetate dihydrochloride (BCE-001) was developed as a CNS stimulant [1] with reference to the structures of the centrophenoxins [2–4] which are used widely in medical practice. The chemical structure of BCE-001 is shown in Fig. 1. In order that a compound may be used as the active material of a pharmaceutical preparation, analytical methods need to be developed for the determination of the active component and for the detection of any contaminants introduced or formed during its production and storage. The present communication details the results of gas and liquid chromatographic studies performed with a view to the measurement of BCE-001 and its impurities.

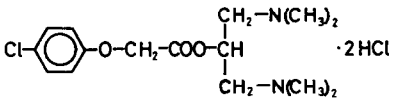

Figure 1
Molecular structure of BCE-001.

Experimental

High-performance liquid chromatography

A Hewlett–Packard (Palo Alto, CA, USA) 1090A liquid chromatograph equipped with a variable volume injector, and a diode array spectrophotometric or a differential refractive index detector (HP 79877A) was used. Hypersil ODS (Shandon Scientific Ltd, Runcorn, UK) 100 × 2.1 mm i.d., 5 μm columns were used along with eluents consisting of a mixture of methanol (HPLC grade from E. Merck, Darmstadt, Germany) and potassium dihydrogenphosphate (pH 2.7, 0.007 M) maintained at a flow rate of 0.5 ml min^{-1} at 40°C. BCE-001 and PCPA were detected spectrophotometrically at 228 or 270 nm. Aliquots of 2–20 μl of 1 mg ml^{-1} solutions were injected.

Gas–liquid chromatography

A Hewlett–Packard 5880 instrument equipped with glass columns and flame ionization detector was employed. The columns (80 cm × 2.1 mm i.d.) were packed with 2% OV-101 (Pierce Eurochemie BV, Oud-Beierland, The Netherlands) on a Chromosorb W-HP 100/200 mesh (E. Merck). Temperature profile: injector at 170°C, oven 80°C (1 min) to 200°C at 10°C min^{-1} (5 min), detector at 250°C. Nitrogen was used as carrier gas at 40 ml min^{-1}. Aliquots of 2 μl of 0.5 mg ml^{-1} solutions were injected.

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Results and Discussion

Reactions of BCE-001 with various alcohols

The main contaminant of BCE-001 is 4-chlorophenoxyacetic acid (PCPA), formed as a result of the hydrolysis of the ester. The UV absorption spectra of BCE-001 and PCPA are superimposable (Fig. 2), thus the two compounds are not readily distinguished spectrophotometrically. On the other hand, after an appropriate separation they may be sensitively detected individually using absorptions at 228 and 270 nm.

Both BCE-001 and PCPA are polar compounds, therefore reversed-phase liquid chromatography was selected as the separation method. The first attempt with a methanol–aqueous KH_2PO_4 mixture (65:35, v/v, pH 2.7) gave a satisfactory separation. At pH 2.7, the dissociation of PCPA is suppressed, and both compounds are eluted as symmetrical peaks. The stability of the analytes in solution was tested by serial injections of a solution containing 1 mg ml^{-1} of BCE-001 and 0.05 mg ml^{-1} of PCPA over a period of 24 h and it was found that no change in the peak areas occurred. However, when the separations were performed under the same experimental conditions, except that the sample was dissolved in ethanol instead of methanol, changes of peak

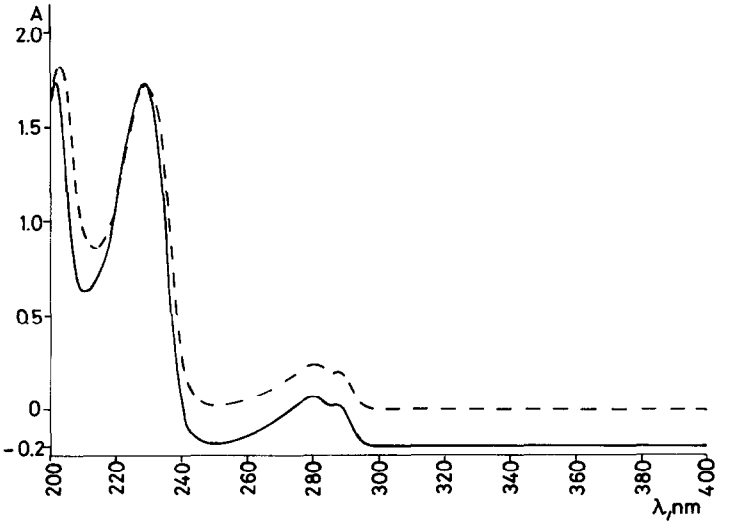

Figure 2
UV spectra of BCE-001 (——) and 4-chlorophenoxyacetic acid (---).

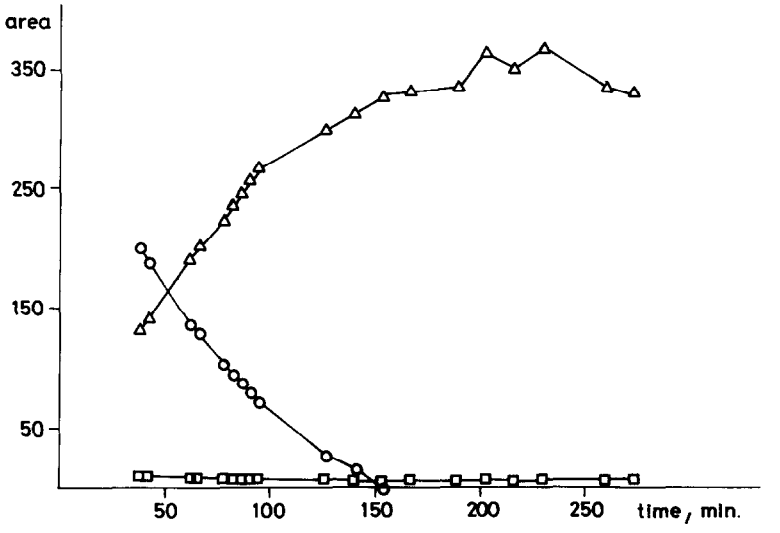

Figure 3
Variation with time of the peak areas observed for samples of BCE-001 dissolved and stored in ethanol. (○), BCE-001 (as PCPM); (□), 4-chlorophenoxyacetic acid; and (△), 'unknown' peak (PCPE). HPLC conditions: a Hypersil ODS column with an eluent consisting of methanol and aqueous KH_2PO_4 (pH 2.7, 0.007 M) (65:35, v/v) at 40°C. Flow rate: 0.5 ml min^{-1}, detection at 228 and 270 nm.

area were observed (Fig. 3). Whereas the peak for PCPA (0.8 min) was unchanged, that of BCE-001 (1.2 min) decreased with time of standing. A new peak appeared at 1.4 min, the intensity of which increased in proportion to the decrease in the area of the BCE-001 peak. As the concentration of BCE-001 approached zero, the area of the new peak reached a constant value.

A possible explanation of this phenomenon is that transesterification of BCE-001 takes place in ethanol, so that the peak eluted at 1.4 min may be ascribed to the ethyl ester of PCPA (PCPE). This finding also suggests that the peak eluted at 1.2 min may be the methyl ester of PCPA (PCPM). The transesterification of BCE-001 to PCPM in methanolic solution takes place much faster than that to PCPE in ethanolic solution.

When BCE-001 was dissolved in isopropanol (only 0.2 mg ml^{-1} could be dissolved in the solvent), it was eluted from the column as PCPM when a methanol-containing eluent was used. The isopropyl ester of PCPA (PCPI) could not be detected in the solution within 1 h.

For identification purposes, PCPI was prepared by dissolving PCPA anhydride in isopropanol and heating it at 80°C under reflux for 2 h. The isolated and purified PCPI was readily soluble in methanol and gave a stable chromatographic peak at 2.8 min. It could be used as an internal standard for the assay of BCE-001. PCPM and PCPE were also prepared via PCPA anhydride in order to obtain unambiguous proof of the behaviour of BCE-001 during its dissolution in methanol and ethanol. Under the same chromatographic conditions, PCPM was eluted at 1.2 min and PCPE at 1.4 min.

Hydrolysis of BCE-001 in aqueous solutions

In aqueous solution, BCE-001, similarly to centrophenoxin (dimethylaminoethyl 4-chlorophenoxyacetate hydrochloride) [5, 6], is hydrolysed to PCPA and presumably to 1,3-bis(dimethylamino)isopropanol (BDIP).

BDIP cannot be detected sensitively by means of a UV detector, therefore a refractive index detector was used for its identification. The composition of the eluent was slightly modified to 62% of methanol and 38% buffer at pH 2.7. The following retention times were observed: 1.78 min for BDIP, 2.12 min for PCPA and 3.28 min for PCPM.

To summarize the experiments described elsewhere, the dissolution of BCE-001 in aqueous methanol or ethanol resulted in two parallel reactions (Fig. 4): transesterification and hydrolysis.

If the rates of hydrolysis and transesterification are compared, it is found that the latter is the higher. This is proved by the fact that when BCE-001 was dissolved in a water–methanol mixture (50:50, v/v), transformation to PCPM was the dominant process and the amount of PCPA, which is a product of hydrolysis, did not increase considerably. The situation was similar when the sample was dissolved in the mobile phase (methanol–water; 65:35, v/v).

On dissolution of BCE-001 in an ethanol–water (50:50, v/v) mixture, the transesterification to PCPE proceeded at a measurable rate, but its hydrolysis to PCPA also occurred in a concurrent reaction (Fig. 5). The following

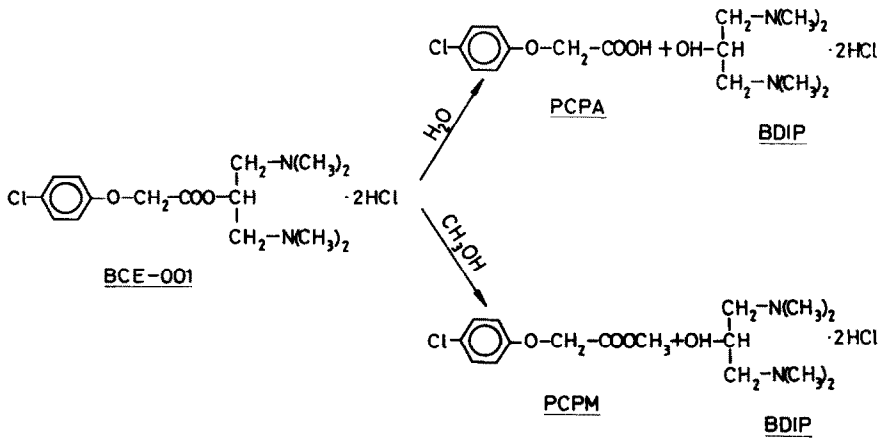

Figure 4
Reactions of BCE-001 with water (hydrolysis) and methanol (transesterification).

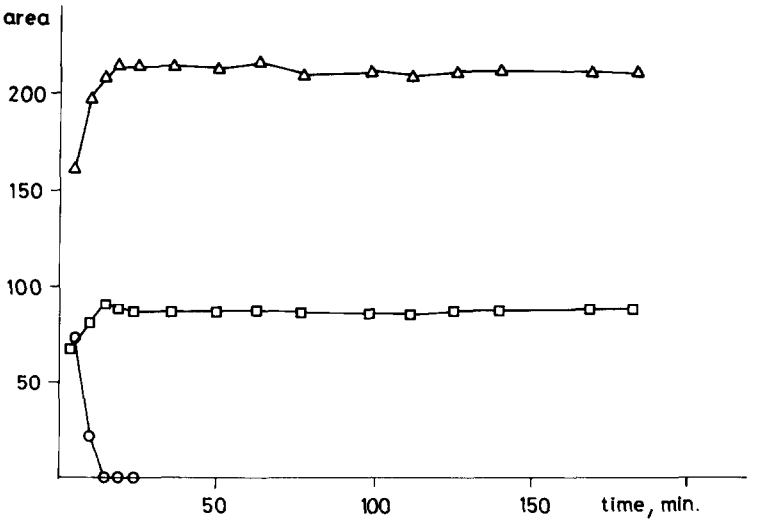

Figure 5
Variation with time of the peak areas observed for samples of BCE-001 dissolved and stored in an ethanol–water (50:50, v/v) mixture. For symbols and HPLC conditions, see Fig. 3.

compounds could be eluted: PCPA at 0.8 min, its amount increasing slightly initially and then becoming constant; PCPM at 1.2 min, its amount decreasing very quickly and PCPE at 1.4 min, its amount first increasing quickly and then becoming constant.

The applicability of liquid chromatography for the assay of BCE-001 is not compromised by the fact that the transesterified derivative of the original compound is measured, provided that the transformation is fast and complete.

Experiments on the direct assay of BCE-001

The transesterification of BCE-001 to PCPM is fast enough, it being complete in about 10 min after the dissolution of the sample, thus peak areas are found to be constant from that time on. The question remained, however, whether the process was complete or had an equilibrium been reached before complete transformation. To answer this question, the original amount of BCE-001 had to be measured. Gas chromatography was chosen for the direct measurement of BCE-001.

A freshly prepared methanolic solution of the compound was injected onto an OV-101 column, which was kept at 80°C for 1 min. The temperature was then increased to 200°C at a rate of 10°C min^{-1} and maintained there for 5 min. PCPM was detected after 6.8 min and BCE-001 after 12.56 min (Fig. 6). PCPA could have been derivatized for direct gas chromatographic measurement, but this would have meant a drastic interference with the system, and therefore was avoided. In a second run,

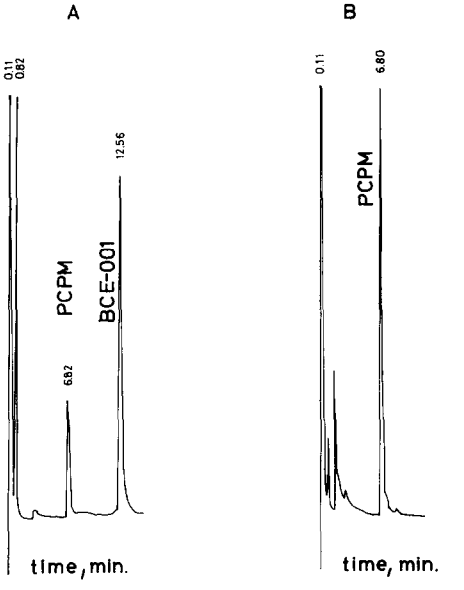

Figure 6
Direct gas chromatographic measurement of BCE-001 and PCPM (A) after dissolving the sample in methanol and injecting immediately; (B) 20 min later. GC conditions: a glass column packed with 2% OV-1 on a Chromosorb W-HP 100/200 mesh. Temperature profile: injector at 170°C, oven 80°C (1 min) to 200°C at 10°C min^{-1} (5 min), FI detector at 250°C. Nitrogen as carrier gas at 40 ml min^{-1}.

carried out 20 min after dissolution of the sample, the intensity of the peak at 6.8 min increased considerably, whilst that at 12.56 min (BCE-001) disappeared. The gas chromatographic measurements confirmed that the transformation of BCE-001 to PCPM was complete if the sample was left to stand in methanolic solution at room temperature for 20 min.

Determination of PCPA content of BCE-001

PCPA may originate as a contaminant of the production process, or it may result from hydrolysis (Fig. 4) taking place during sample pretreatment. This decomposition occurs even in the solid state during long-term storage, although it can be suppressed considerably if the water content of the product is decreased to a minimum level and the uptake of moisture is prevented.

The knowledge gained concerning the transformations of BCE-001 in aqueous–methanolic solutions necessitated a re-examination of the liquid chromatographic method for assaying the PCPA content of BCE-001 samples. Pure PCPA was dissolved in methanol and methanol–water mixtures of various compositions, and the intensities of the resulting chromatographic peaks measured as a function of time. No changes were found, indicating that there is no reaction between PCPA and methanol or water, although the peak shapes recorded for the methanolic solutions became slightly distorted with time.

BCE-001 samples for analysis were dissolved in methanol (65% v/v of the final volume); then, after differing time intervals, the solution was made up to the final volume with water and injected immediately. The results for a given sample which contained a relatively large amount of PCPA are listed in Table 1. The PCPA content of the sample did not change after 30 min; it was then 3.24%. The higher values measured at shorter times are due to the hydrolysis of part of the BCE-001 to PCPA, since water was added to the solution before completion of the transesterification to PCPM. If the sample was dissolved in the eluent (methanol–water; 65:35, v/v) and injected immediately, the PCPA content was found to be 6.13%.

These results clearly show that an unsuitable sample pretreatment can cause serious errors. It was proved unambiguously that the transesterification was complete in 30 min in methanolic solution; on the addition of water to the solution, therefore, hydrolysis could not take place (PCPM is much more resistant to hydrolysis) and the amount of PCPA did not increase further. Gas chromatography did not reveal any BCE-001 after 20 min, but PCPA could not be measured by this method.

When the same liquid chromatographic method was used for the determination of PCPA as that developed for the assay of BCE-001, with a modified sample pretreatment, the detection limit was 10 ng PCPA (measured at 228 nm); that is, 0.05% PCPA could be measured when 20 µl of BCE-001 sample was injected.

Table 1
Changes in PCPA content of a BCE-001 sample as a function of time between addition of methanol and water to the samples (see text). For HPLC conditions, see Fig. 3, detection at 228 nm

Time (min)	PCPA (%)
1	6.03
5	5.29
10	4.21
20	3.34
30	3.24
40	3.24

Acknowledgements — The authors thank the Chemical Research Department of BIOGAL for their help in the preparative work, and Mrs Mária Kiss for valuable assistance in the experimental work.

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[*Revised manuscript received 26 September 1991*]