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Duration of effect of the mouthwash CB12 for the treatment of intra-oral halitosis: a double-blind, randomised, controlled trial

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Abstract

Halitosis occurs in approximately 30% of the adult population and has a negative social and psychological impact on affected individuals. Mouthwashes may be used to prevent unpleasant odour, with long-duration of effect being a desirable attribute. The aim of this study was to assess the long-term efficacy of CB12 (a mixture of 0.3% zinc acetate and 0.025% chlorhexidine) for the treatment of intra-oral halitosis. Thirty-four subjects with confirmed intra-oral halitosis were randomized into a double-blind, controlled, cross-over study to one of 2 groups; (i) CB12–water–water or (ii) water–CB12–CB12. Each group comprised 3 treatments, each given evening and morning (12 h apart) on consecutive study days, with a 5 d washout between treatments. Intra-oral halitosis was assessed objectively by measuring concentrations of hydrogen sulphide, methyl mercaptan, dimethyl sulphide and total volatile sulphur compound (VSC) concentrations and subjectively using organoleptic score (OLS). These were measured at baseline, 12 h after the evening rinse (i.e. 12 h overnight assessment) and 12 h after the daytime rinse (i.e. 12 h day time assessment). CB12 significantly reduced mean hydrogen sulphide, methyl mercaptan, dimethyl sulphide and VSC concentrations, with a duration of effect lasting 12 h, whether assessed overnight (all $p \leqslant 0.0003$ versus water) or during the day (all $p \leqslant 0.0007$ versus water). CB12's effect on OLS was also evident for 12 h overnight ($p = 0.0043$). CB12 was well-tolerated. In conclusion, CB12 showed a clear and durable effect on intra-oral halitosis which lasted at least 12 h, both during the day and overnight, with consistent effect on both objective and subjective variables.

Introduction

Halitosis is a general term used to describe an unpleasant odour from the breath. In around 90% of cases the problem is attributable to volatile sulphur compounds (VSC) produced by anaerobic bacteria in the oral cavity [1], a condition that has been defined as intra oral halitosis [2]. The VSCs found in the mouth are mainly hydrogen sulphide, methyl mercaptan and dimethyl sulphide, and these have an unpleasant odour even in small concentrations [1]. Epidemiological evidence on persistent halitosis is limited and difficult to evaluate due to a lack of uniformity in measurement and diagnosis, and the data is often based on self-estimation, limiting its accuracy [3]. However, the prevalence of halitosis in the general public is estimated at around 30% [4–7].

Organoleptic scoring by a trained odour judge is considered the gold standard for assessing breath odour [3, 8–12]. Though subjective and hard to standardise, the organoleptic score (OLS) is a direct measure of the extent of halitosis, and hence is the measure of most importance to the patient [2]. Commercially available devices such as the OralChroma gas chromatograph and Halimeter provide objective measures of VSC, known to be the principal component of breath odour,

and have been shown to correlate well with organoleptic assessment [12–14].

Recommended treatment needs for halitosis have been defined by an international consensus group based on earlier publications [2, 15, 16]. As well as professional treatments, home-based oral hygiene measures including tongue cleaning and the use of antibacterial mouthwashes are recommended [2]. A recent systematic review of the literature by Blom *et al* [17] concluded that nearly all mouthwashes with active ingredients helped to reduce bad breath, but the most compelling evidence was for those containing chlorhexidine, cetyl pyridinium and zinc. Mouthwashes containing a combination of zinc ions and chlorhexidine have been shown to be particularly effective in inhibiting the formation of VSCs [18–21]. However, mouthwashes containing high concentrations of chlorhexidine have been associated with a number of unwanted side effects such as irritation to oral mucosa, burning sensations, tooth staining and unpleasant or altered taste, precluding their long-term use [18, 22, 23].

CB12 (MEDA OTC, Sweden) is a commercially available, over-the-counter mouthwash, containing a combination of two active ingredients: zinc acetate (0.3%) and a low concentration of chlorhexidine (0.025%). Previous studies with CB12 have shown it to be effective in treating halitosis by neutralising hydrogen sulphide, methyl mercaptan and dimethyl sulphide [19, 24, 25]. Thrane *et al* [19] tested CB12 in 19 healthy volunteers in a non-randomised, one way cross-over, open label trial and found significantly greater inhibition of VSC production compared with rinsing with water. A four-week follow-up of a sub-group of subjects in the same study found that daily use of CB12 did not cause tooth discolouration or other side effects such as mucosal lesions or taste disturbance. In a double blind, 6-fold crossover study in 14 healthy adults with a baseline OLS $>$ 2 on the study morning, CB12 showed clear superiority in improving OLS and reducing mean VSC levels versus other mouthwashes and water over a short observation period (between 30 min and 3 h) [24]. A more recent trial found no halitosis (identified by VSC assessment) at day 14 in 12 out of 21 subjects using a zinc and chlorhexidene rinse, compared with 1 of 21 using a negative control rinse [25].

The present study was conducted in order to evaluate the duration of effect of CB12 within the rigorous setting of a randomised, controlled, double-blind trial in subjects with intra-oral halitosis under highly standardized conditions.

Methods

The study was conducted in agreement with the following directives, laws, and guidelines: the Declaration of Helsinki; ICH guideline on Good Clinical Practice (ICH E6); EU Cosmetic Products Regulation No 1223/2009; and The Scientific Committee on Consumer Safety's Notes of Guidance for Testing of Cosmetic Ingredients and their Safety Evaluation [26]. The study was ethically approved in accordance with German regulations.

Study population

Participants were recruited from dental study sites in Germany with a halitosis out-patient department. Subjects were eligible for inclusion if they were otherwise healthy, but had halitosis of intra-oral origin with daily periods of noticeable halitosis. They were required to have an OLS $\geqslant$ 2 (Rosenberg Score adapted by Greenman) [11] prior to the first dose of study treatment of each study period. Subjects were also required to have a total VSC concentration $>$160 ppb, a hydrogen sulphide concentration $\geqslant$112 ppb and a methyl mercaptan concentration $\geqslant$26 ppb prior to the first dose of study treatment at the first study period. Written informed consent was obtained from all subjects prior to enrolment.

Individuals were excluded from the study if they had a history of reactions to alcohol or any of the ingredients of study treatment; extra-oral halitosis, as indicated by lack of significant difference between the results of organoleptic assessments from nasal and oral breath; periodontitis with a periodontal screening and recording index $>$Code 2 in more than one sextant [11]; open caries lesions $>$D2; obvious gingival inflammation, gingivitis or advanced periodontitis; oral thrush; history of malignant or infectious diseases; systemic medication associated with oral dryness within 1 month prior to, or during, the study period; use of mouth care products containing chlorhexidine or zinc compounds $<$2 weeks before first study treatment; or systemic antibiotic therapy within the preceding 3 months.

Study treatments

CB12 contains 0.3% zinc acetate dihydrate, 0.025% chlorhexidine diacetate, aqua, glycerin, hydrogenated starch hydrolysate, alcohol, sodium fluoride, PEG-40 hydrogenated castor oil, potassium acesulfame, citric acid and aroma. The control solution contained non-carbonated water (Acqua Panna, Nestlé, Germany) filled into clean empty CB12 bottles provided by, and relabelled at, MEDA (Radebeul, Germany). All treatments were administered by rinsing 10 ml in the mouth for 30 s, corresponding to the user instructions for CB12.

Study protocol

The study had a randomized, double-blind, controlled, cross-over design. Subjects were randomised 1:1 to one of two groups, with three treatment periods in each group; Group 1: CB12-water–water and Group 2: water-CB12–CB12. The second period of each sequence was replicated to estimate a potential carry over effect, and there was a 5 d washout between treatments.

Oral concentrations of hydrogen sulphide, methyl mercaptan and dimethyl sulphide were measured

using an OralChroma gas chromatograph (ABI Medical). Total concentration of oral VSCs was assessed using a RH-17 Halimeter (Ansyco). Both the Halimeter and OralChroma were calibrated by the respective manufacturer prior to the study. The intensity of bad breath was assessed by a trained odour judge using the 0–5 organoleptic scale by Rosenberg (modified by Greenman): 0 = no odour; 1 = barely noticeable odour; 2 = slight odour; 3 = moderate odour; 4 = strong odour; 5 = very strong odour (saturation) [27, 28]. All assessments were made at baseline, 12 h after the first rinse following a night's sleep (i.e. 12 h overnight effect), and 12 h after the second rinse (i.e. 12 h daytime effect). Total VSC concentration was also assessed at screening. A single reading was taken at each time point for OralChroma assessments. Three readings were taken at each time point for Halimeter assessment and the median recorded.

Environment standardization

All study treatments were administered under supervision of the investigator's staff, so separate compliance assessment was not required. To enable investigators to be blinded during assessments they were not allowed to be present during administrations. Subjects were present at the study site from early evening, approximately 3 h (for Period 1 approximately 27 h) before first administration and up to 14 h after second administration of study treatment within each period, and stayed on the study ward from the time of admission to the last measurement. Subjects were instructed to refrain from their usual dental and mouth hygiene procedures on the day of admission. No changes in oral hygiene practices were permitted during the study and the consumption of foods associated with oral malodour (such as garlic, onions or alcohol) was not allowed on study days or in the preceding 48 h. Smoking was not allowed during the study period, and consumption of sweets or lozenges containing agents with impact on the oral microbiota (e.g. antimicrobial or breath-refreshing effect) was not allowed on screening visit or study days, or in the preceding 48 h. Subjects were also asked to fast for at least 3 h before admission to the clinical unit and VSC baseline assessments, for at least 12 h from first dosing to VSC assessments and organoleptic scoring in the morning and for at least 3 h prior to the evening VSC assessments and organoleptic scoring.

Meals and amount of beverages were standardized throughout the study. Subjects received a standardized meal prior to the first mouth rinse, a breakfast after morning assessments prior to the 2nd mouth rinse, lunch at approx. 4 h and a snack 8 h after the 2nd mouth rinse. Moreover, intake of 500 ml non-carbonated water was allowed from 1 h after the first mouth rinse until 3 h before morning assessments, and a further 1500 ml permitted from 1 h after the second mouth rinse to 3 h before evening assessments following the 2nd mouth rinse. No other beverages were permitted.

Safety

All adverse events (AEs) were recorded and classified. AEs were considered treatment emergent (TEAE) when occurring from the first administration of study treatment (0 h) until at latest 24 h after the second administration of study treatment. AEs occurring or worsening after that period were not analysed as TEAEs, but were considered separately as AEs.

Statistical analysis

Based on effect size and variances from preliminary studies [19, 20] it was estimated that 17 subjects per group would be required to achieve an overall power of 95% (probability to achieve the two primary and the key secondary endpoint—see below) with a one-sided α level of 2.5%. A drop-out rate of 15% was assumed. The intention-to-treat (ITT) population was defined as all subjects randomised and exposed to study treatment who had at least one follow-up assessment of efficacy. The per-protocol-population was a subset of the ITT population excluding subjects with major protocol violations. The ITT was the primary population for all study end points.

The primary efficacy measures were change in hydrogen sulphide and methyl mercaptan concentrations from baseline to 12 h after the first rinse with either CB12 or water (i.e. 12 h overnight effect). The key secondary outcome measure was change in OLS over the same time period.

Each of these endpoints were tested hierarchically in that order to maintain an overall one-sided type I error level at $\alpha = 2.5\%$. Logarithms of 12 h measurements of the primary variables were analysed by baseline-adjusted ANCOVAs, with treatment, treatment sequence, period and carry-over (effect of prior period) as fixed effects, and the logarithm of the period baseline as a continuous covariate. Due to the use of log-transformation, 0-values were replaced with 1 as the lowest detectable value by the chromatograph. The covariance matrix among periods was left unspecified and was allowed to vary among treatment sequences. Satterthwaite approximation was used for the degrees of freedom. Missing values were not replaced. OLS was analysed with the same ANCOVA model but without logarithmic calculus and baseline adjustment.

Primary and key secondary efficacy variables assessed 12 h after the second rinse (i.e. 12 h daytime effect) were classified as secondary efficacy outcomes. Other secondary efficacy variables were dimethyl sulphide and VSC concentrations 12 h after each mouth rinse (i.e. both overnight and daytime effect). All of these secondary variables were analysed by the same ANCOVA model as described for the primary efficacy parameters. Safety analyses were carried out for all randomised subjects who received at least one dose of study treatment (the safety population).

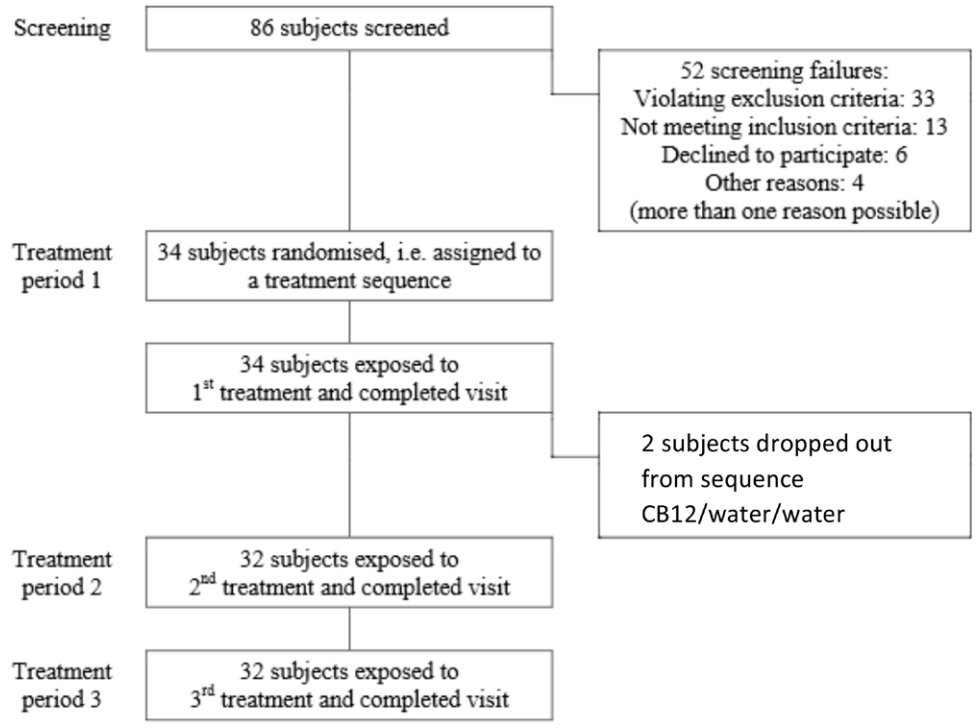

Figure 1. Subject disposition.

Results

Subject disposition and characteristics

A total of 86 subjects were screened and 34 were included in the study (figure 1). Two subjects were withdrawn from the study after the first treatment period, one for an adverse event (respiratory infection) and one for non-adherence to study procedure. Both were excluded from the per protocol population (but not from the ITT population). No other major protocol deviations occurred.

Subject baseline characteristics are presented in table 1. Continual mouth odour was reported in 12 (35.3%) subjects, 10 (29.4%) reported problems in the morning and evening and 12 (35.3%) reported problems in the morning only. Almost all subjects (97%) already used mouth odour products. Subjects had variable degrees of tongue coating (mean Winkel score/index 4.3) and caries (mean DMF-T index 12). Mean VSC concentrations at screening were 208.6 ppb in treatment group 1 and 300.8 ppb in Group 2 (table 2). The difference was largely due to one subject who had an extremely high value (1572 ppb).

Volatile sulphur compounds

Compared to water, CB12 provided a significantly greater reduction in oral concentrations of hydrogen sulphide (Diff: −1.409; 95% CI −1.826, −0.993; $p < 0.0001$; figure 2(A)) and methyl mercaptan (Diff: −1.054; 95% CI: −1.420, −0.688; $p < 0.0001$; figure 2(B)) 12 h after the first rinse (i.e. overnight effect). Subjects who rinsed with water experienced marked overnight increases in oral concentrations of both hydrogen sulphide and methyl mercaptan, which was not the case with CB12 (figures 2(A) and (B)). The effect on both of these variables was still present 12 h after the second treatment, with CB12 use associated with a significantly greater reduction in oral concentration of hydrogen sulphide (Diff: −1.706; 95% CI: −2.257, −1.155; $p < 0.0001$) and methyl mercaptan (Diff: −1.566; 95% CI: −2.200, −0.933; $p < 0.0001$) compared to water (figures 2(A) and (B)).

CB12 also induced a significantly greater reduction in oral dimethyl sulphide concentration compared to water both 12 h after the first rinse (Diff: −0.757; 95% CI: −1.153, −0.356; $p = 0.0003$) and 12 h after the second rinse (Diff: −0.849; 95% CI: −1.335, −0.362; $p = 0.0007$) (figure 2(C)). Similarly, CB12 induced a significantly greater reduction than water in total VSC concentration for both the 12 h overnight assessment (Diff: −0.503; 95% CI: −0.655, −0.352; $p < 0.0001$) and the 12 h daytime assessment (Diff: −0.509; 95% CI: −0.676, −0.342; $p < 0.0001$) (figure 2). For each variable the three treatment periods showed similar time courses.

Organoleptic assessment

Subjects who rinsed with CB12 experienced a significantly greater 12 h overnight reduction in mean OLS than those who rinsed with water, across the three treatment periods (Diff: −0.5; 95% CI: −0.9, −0.1; $p = 0.0042$). CB12 subjects experienced an OLS reduction from 3.0 (SD 0.6) at baseline to 2.5 (SD 0.7) 12 later. Conversely, subjects who rinsed with water experienced no reduction in OLS 12 h later, remaining at their baseline OLS score of 2.8 (SD 0.7).

Table 1. Subject baseline characteristics.

Sex, *n* (%)	Male 17 (50)
Age, mean (range), years	44.2 (22–73)
BMI, mean (range), kg m^{-2}	25.8 (18–38.6)
DMFT index, mean (range)	12 (0–23)
Total Winkel score, mean (range)	4.3 (2–10)
Dietary variables, *n* (%)	
Coffee drinkers	28 (82.4)
Non-coffee drinkers	6 (17.6)
Light alcohol drinkers	24 (70.6)
Non-drinkers	10 (29.4)
Daily meat consumption	9 (26.5)
Occasional meat consumption	18 (52.9)
Vegetarian/vegan	2 (5.8)
Concomitant treatment, *n* (%)	11 (32.4)
Analgesics[a]	3 (8.8)
Thyroid therapy	3 (8.8)
Anti-inflammatory/rheumatoid products	2 (5.9)
Current use of products for mouth odour, *n* (%)	33 (97)
Mouth rinse products	23 (67.7)
Chewing gum	23 (67.7)
Dental floss	19 (55.9)
Lozenges	17 (50)
Mouth odour history, *n* (%)	
Continuous mouth odour	12 (35.3)
Problems in morning and evening	10 (29.4)
Problems in the morning only	12 (35.3)

BMI: body mass index; DMFT: decayed, missing, and filled teeth.
[a] Only reported where incidences >5%.

Table 2. Screening with the Halimeter for included subjects.

		VSC concentration (ppb)	
Treatment sequence	*N*	Mean (SD)	Median (IQR)
CB12/Water/Water	18	208.6 (100.00)	168.3 (276.0, 479)
Water/CB12/CB12	16	300.8 (346.13)	218.8 (248.0, 1572)
All	34	252.0 (248.58)	188.5 (265.5, 1572)

VSC: volatile sulphur compound; ppb: parts per billion; SD: standard deviation; IQR: inter-quartile range.

CB12 induced continued improved in OLS 12 h after the second rinse (i.e. 12 h day time effect), reducing the OLS to 2.4 (SD 0.6), compared to 2.6 (SD 0.8) for those in the water group (Diff: −0.3; 95% CI: −0.7, 0.1; $p = 0.0458$). The OLS assessments in this study were not very discriminative (baseline measurements showed values between 2 (lowest value) and 4 (highest value) leading to low correlations of objective and subjective assessments (R 0.48 for VSC Halimeter/OLS; R 0.24 for H_2S OralChroma/OLS).

For all variables assessed, broadly similar findings were found irrespective of when intra-oral halitosis was predominantly experienced (i.e. continuously, morning & evening, or morning only; data not shown).

Table 3. Adverse events.

	CB12 ($N = 34$) *n* (%)	Water ($N = 32$) *n* (%)
Serious AEs	0 (0)	0 (0)
Treatment-emergent AEs		
Application site reaction	1 (2.9)	0 (0)
Dysgeusia	1 (2.9)	0 (0)
Fatigue	1 (2.9)	0 (0)
Headache	4 (11.8)	4 (12.5)
Vomiting	0 (0)	1 (3.1)

AE: adverse event.

Safety

Six subjects reported a TEAE following CB12, and five subjects following water (table 3). Two of the TEAEs were considered to be related to CB12 administration: application site reaction and dysgeusia ($n = 1$ each; 2.9%). The most frequently reported TEAE was headache, reported by four subjects following CB12 and four subjects following water. No causal relationship between the occurrence of headache and the administration of the study treatments was seen, and headache was assessed as unlikely to be an adverse reaction. Vomiting was reported in one subject following water. There were no serious AEs. One subject discontinued treatment due to a wisdom tooth extraction.

Discussion

Halitosis can cause a significant social burden [6, 29, 30]. Satisfactory treatment, such as use of anti-bacterial mouthwashes, is considered beneficial to combat bad breath and has a positive impact on both social life and self-confidence [2, 31]. From a clinical perspective the duration of the VSC-reducing effect is of high interest. Our aim was to assess the long-term efficacy of CB12 in a well-controlled clinical trial setting using both objective and subjective measures of intra-oral halitosis. The results showed that CB12 effectively combated halitosis, and reduced the VSCs associated with it, for up to 12 h.

For the testing of VSC-reducing measures in breath malodour research, Yaegaki *et al* recommend a short-term experiment in a cross-over design [15]. Based on their research they concluded that short-term studies are preferable in terms of standardisation because oral levels of VSCs show circadian variations [32] and are influenced by a variety of factors such as eating. However, short-term testing has no or limited ability to predict the long-lasting effect of a treatment, nor the cumulative effects of multiple treatments. For the current study, a highly standardized environment was chosen. All participants were treated, and then stayed in the study ward for 24 h under the supervision of study staff, with controlled meals. Study procedures were performed in accordance with a protocol that reflected

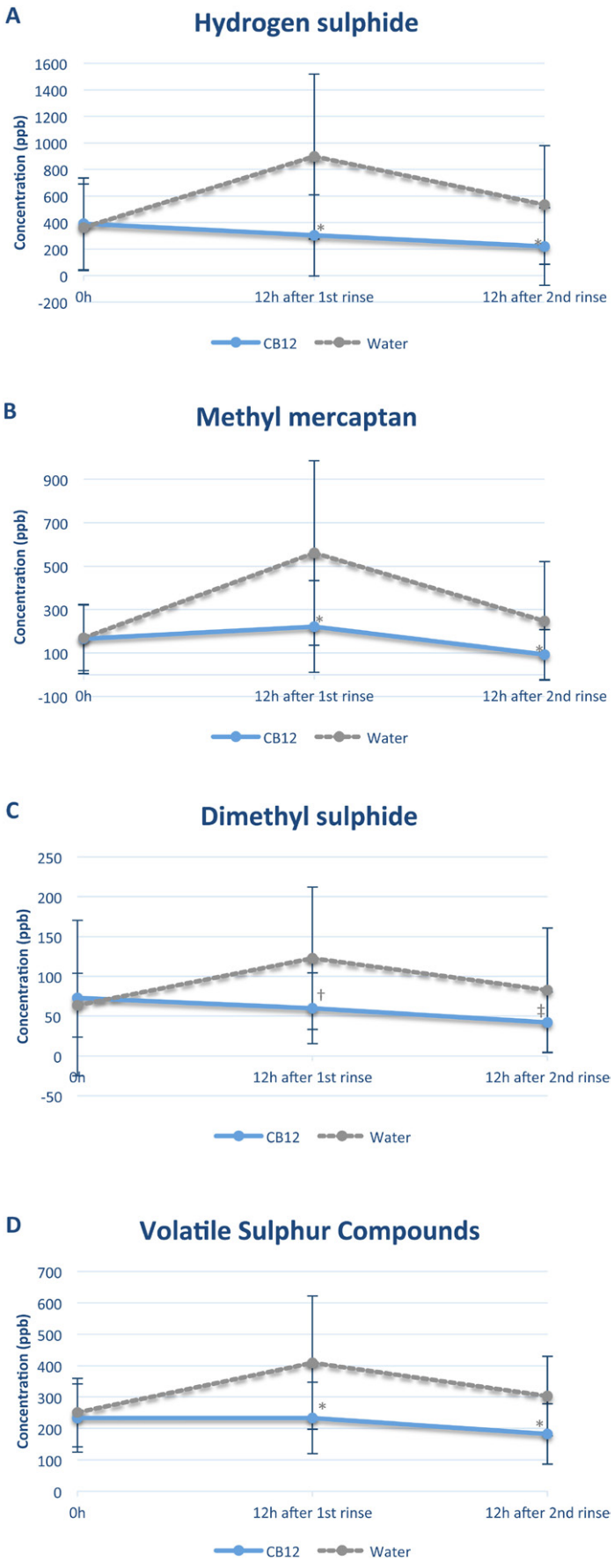

Figure 2. (A) Hydrogen sulphide, (B) methyl mercaptan, (C) dimethyl sulphide and (D) total VSC concentrations at baseline, 12 h after first rinse and 12 h after 2nd rinse of CB12 or water in subjects ($n = 34$) with halitosis of intra-oral origin. $^{*}p < 0.0001$ versus water; $^{\dagger}p = 0.0003$ versus water; $^{\ddagger}p = 0.0007$ versus water.

instruction-compliant real-world use, and the study was carried out to a rigorous randomized controlled trial design, and in line with Good Clinical Practice requirements. Other influencing factors were also tightly controlled.

In the current study, a clear, durable and statistically significant effect on intra-oral halitosis after instruction-compliant use of CB12 (i.e. morning and evening rinse) was observed for all primary and key secondary endpoints. CB12 showed significantly greater efficacy over water in reducing oral levels of hydrogen sulphide, methyl mercaptan, dimethyl sulphite and total VSC concentrations, with a 12 h duration of effect. This effect was apparent when assessed overnight (i.e. 12 h after the first rinse) and during the day (i.e. 12 h after the second rinse), thus confirming that 24 h breath-odour control is achievable with instruction compliant use of CB12. The overnight 12 h duration of effect of CB12 is particularly relevant for sufferers, as maximum intensity of halitosis can occur in the mornings due to a reduction in saliva flow combined with an accumulation of VSCs due to bacterial re-growth overnight; a problem for approximately 65% of subjects in this study. A confirmed 12 h duration of effect during the day-time is also relevant for individuals with intra-oral halitosis, providing confidence of fresh breath during the day, when social interactions are maximised.

For the organoleptic assessment of halitosis performed by an experienced judge, a statistically significant difference ($p = 0.0043$) was observed between the CB12- and water-treated subjects for the 12 h overnight assessment, the key secondary efficacy parameter. Water-treated subjects showed increased morning values, whilst CB12-treated subjects had decreased scores. Again, these results are of particular importance to those subjects who reported morning bad breath. However, the absolute reduction in OLS showed that halitosis was present after 12 h, and the reduction compared to baseline appeared small. The study design did not allow for any judgement on the development of halitosis levels from baseline (directly after application up to the end of the 12 h period), or on the effect after 12 h, but it can be assumed that higher reductions were present in this period.

Instrumental measures have been widely included in the diagnostic procedures for halitosis. OralChroma gas chromatography provides an objective measure of halitosis and has shown good correlation with OLS in former investigations [13, 14]. However, as OLS assessments in this study were not very discriminative, the correlations of objective and subjective assessments were low. The Halimeter is also used as a measure of halitosis, but is limited by its insensitivity to mid- to low-level concentrations of VSCs; this sensitivity is even lower for dimethyl sulphide [13, 33]. Dimethyl sulphide levels do not always correlate well with OLS [13, 33]; thus dimethyl sulphide was chosen as a secondary variable in the current study.

The short-term effect of CB12 has been confirmed in previous studies [20, 21, 24, 25]. These studies showed CB12 to be effective in the reduction of halitosis, and superior to both placebo and a number of different mouthwashes for a range of halitosis-related outcome measures. The results of the present study now confirm the long-term effect of CB12 in a robust and well-controlled study design. How CB12 maintains its 12 h duration of effect is a subject of debate. Its active ingredients, zinc acetate and chlorhexidine, operate by different mechanisms; Zinc interacts with the sulphur in the substrate or in precursors of VSC, forming insoluble sulphides (since it has an affinity for sulphur and oxidises sulfhydryl groups [34]) and may also directly inhibit thiol proteinase activity related to VSC production [35]. Chlorhexidine is known to be a strong denaturing agent. A splitting of disulphide bonds would be beneficial as oral bacteria mainly contain desulphydrases [36]. The splitting of disulphide bonds could provide an explanation for the observed marked and long-lasting effect of CB12. Furthermore, Young and colleagues [36] showed a synergy between zinc and chlorhexidine; this combination provided a reduction of >95% of the baseline VSC concentration even 9 h after rinsing. The authors concluded that under normal conditions (without cysteine challenges) it may be safe to conclude that the mouth rinse (CB12) could be effective for 12 h or more [36]. Indeed, Thrane *et al* [19] showed that CB12 effectively inhibited oral VSC production for over 12 h, both with and without cysteine challenge, 'likely due to a synergistic effect of zinc and chlorhexidine on VSC', further corroborating the results of the present study.

CB12 was well tolerated; the only AEs considered likely to be adverse drug reactions were dysgeusia and application site reaction in one subject each. Headache was reported in 11.8% of subjects following CB12 and 12.5% following water. Dietary restrictions in this study included refraining from all xanthine-containing beverages and food (e.g. coffee, black and green tea, cola, any chocolate) for the entire duration of the in-house stay. It is therefore possible that the headaches were a result of caffeine withdrawal.

A potential limitation of the study was that CB12 was compared with water. There is a possibility that subjects could distinguish the two treatments by taste. However, this is unlikely to have led to bias as the odour judges were fully blinded, and subjects were told not to communicate impressions of smell and taste to ensure that blinding was preserved. Recruitment of individuals with confirmed intra-oral halitosis was a strength of this study, unlike others which have often relied on volunteers with morning breath or used cysteinyl challenge. Indeed, the strict inclusion and exclusion criteria relating to intra-oral halitosis explain the relatively high screen failure rate in our study (60.5%). Additionally, in the current study all efforts were made to standardize the environment, removing as much variability as possible, in order to prove a direct cause and effect relationship between CB12 use and intra-oral halitosis abatement for 12 h. Finally, results obtained from the gold-standard subjective measure of intra-oral halitosis assessment (i.e. OLS) were confirmed using objective VSC assessments.

In conclusion, this study confirms that rinsing with CB12, in accordance with its instructions for use, led to a statistically significant reduction in intra-oral halitosis, assessed both subjectively and objectively, with an effect lasting for 12 h. Use of CB12 twice daily (morning and evening) provided 24 h protection from VSC production associated with intra-oral halitosis.

Conflict of interest

The authors have no conflict of interest.

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