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Food Interactions Observed in a Pharmacokinetic Investigation Comparing Two Marketed Cold Preparations (BNO1016 and ELOM-080) after Administration to Beagle Dogs – A Pilot Study

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ABSTRACT

Sinupret extract (BNO 1016) and Gelomyrtol forte (ELOM-080) represent the two top-selling cold remedies in Germany nowadays. Whereas BNO 1016 is a typical immediate release coated tablet, ELOM-080 is an enteric-coated soft gelatin capsule. The latter formulation, however, is at risk of pharmacokinetic interactions affecting absorption, especially in cases of concomitant food intake. In the present pilot study, we investigated the risk of a possible food effect in three male beagle dogs. Single doses of BNO 1016 and ELOM-80 were administered under fasting and fed conditions. Blood was sampled up to 30 h post-administration and plasma concentrations of the characteristic ingredients of BNO 1016 as well as ELOM-080 analytes were determined. Pharmacokinetic parameters focusing on the rate and extent of absorption were derived. BNO 1016 analytes demonstrated a similar course in both the fasted and fed states. ELOM-080 analytes also showed a similar picture in the fasted state. However, lag times (time from administration to first quantifiable time point in plasma) of up to 2 h post-administration with corresponding time to reach maximum concentration (obtained directly from the measured concentration) values of 3 to 4 h were observed, reflecting a longer gastric residence time. In the fed state, ELOM-080 showed significant pharmacokinetic characteristics, suggesting a clear food effect. A major observation was a double peak phenomenon that could be observed in two of three dogs. Furthermore, lag times of some analytes, up to 3–4 h, and corresponding time to reach maximum concentration values, up to 6–8 h, occurred. In contrast to BNO 1016, these findings suggest that, as with other enteric-coated formulations, there may also be a significant risk for food effects with ELOM-080 in humans.

Introduction

Today, the spectrum of phytopharmaceutical preparations ranges from immediate release tablets, capsules, and lozenges to effervescent tablets, solutions, syrups, and teas. It is noticeable that modified release or sustained release dosage forms obviously do not play a significant role in phytotherapy, although therapeutically, they could provide a good benefit, especially in some chronic indications [1, 2]. Looking at herbal cold preparations, products for the treatment of sinusitis/rhinosinusitis and acute or chronic bronchitis dominated the market in Germany in 2020 [3]. The two top-selling cold remedies (R05C class) in pharmacies were Sinupret extract (BNO 1016) and Gelomyrtol forte (ELOM-080), with around 3 million packs sold. They are both also marketed in-

ABBREVIATIONS

API	active pharmaceutical ingredient
$AUC_{(0\text{-tlast})}$	area under the plasma concentration vs. time curve from dosing time to the last measurement time point with a concentration value above the lower limit of quantitation, calculated by means of the linear/log trapezoidal method, which uses the linear trapezoidal rule up to C_{max} and afterwards the $\log_{(interpolation)}$ trapezoidal rule for the subsequent part of the curve
C_{max}	maximum concentration in plasma (taken directly from the measured concentration values)
EMA	European Medicines Agency
LLOQ	lower limit of quantification
p. a.	post-administration
QC	quality control
SmPC	summary of product characteristics
$t_{1/2}$	apparent terminal elimination half-life
t_{lag}	time from administration to first quantifiable time point in plasma
t_{max}	time to reach maximum concentration (obtained directly from measured values)

ternationally and in member states of the European Union. Surprisingly, ELOM-080 is a modified release dosage form, more precisely an enteric-coated soft gelatin capsule. In general, the purpose of enteric-coated medicines is either to protect the contained drug from, e.g., degradation until it is released in the intestine, or to protect the stomach from damage by the drug. One reason for the dosage form of ELOM-080 could be that it is a preparation with essential oil components that perfectly fit to soft gelatin capsules. The special essential oil distillate ELOM-080 [4] has been shown to activate mucociliary clearance [5–7]. This has also been proven for BNO 1016, an immediate release coated tablet formulation [8–10] containing an extract of cowslip flowers, yellow gentian root, black elderberry flowers, sorrel herb, and verbena herb [11]. Moreover, nonclinical data have demonstrated its anti-inflammatory activity [12]. Flavonoid compounds are being discussed as the active ingredients. Since both preparations are used for acute infections of the respiratory tract, the dosage forms should be designed in such a way that a rapid release of the active substance and a rapid therapeutic effect can be achieved.

Modified release or enteric-coated oral dosage forms, however, are at risk of pharmacokinetic interactions affecting the absorption process, especially in the case of concomitant food intake [13–15]. Such food effects are often not predictable from the *in vitro* characteristics of a dosage form. Several investigations have demonstrated a high probability of food interactions for formulations with pH-dependent release properties, such as enteric-coated formulations [16], which present a quite sizeable, indigestible solid particle. Passage of such particles through the pylorus occurs normally during phase III of the migrating motor complex. Food intake interrupts the migrating motor complex and can thereby delay the passing of enteric-coated formulations from the stomach into the duodenum [17, 18]. Therefore, an *in vivo* food interaction might also be suggested to occur with the intake of ELOM-080 enteric-coated capsules.

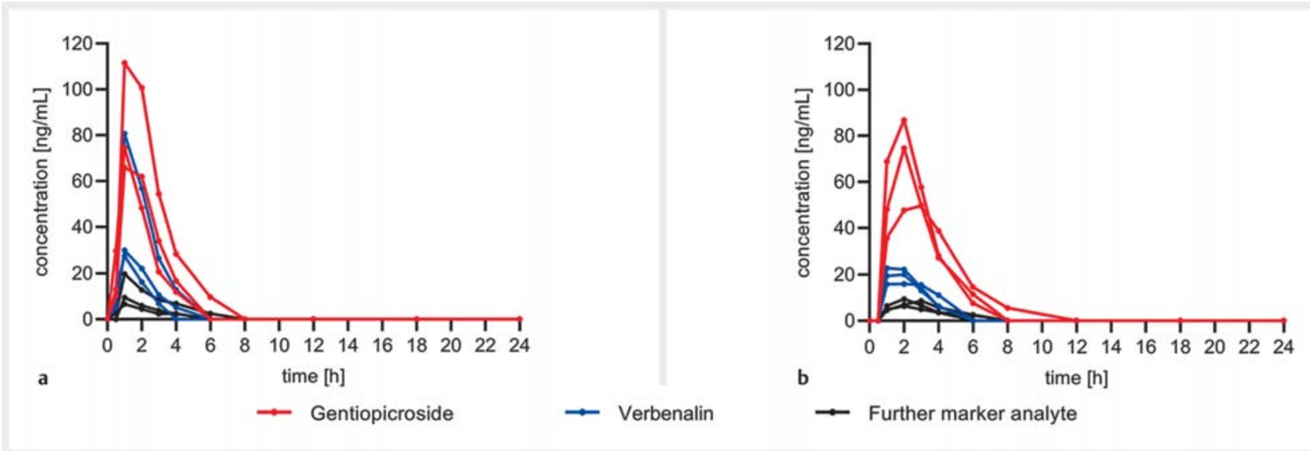
In the present exploratory pilot study, we compared possible effects of food intake on ELOM-080 enteric-coated capsules and BNO 1016 immediate release coated tablets in male beagle dogs. Beagle dogs are considered a standard model to predict possible food effects of drug formulations [18, 19].

Results

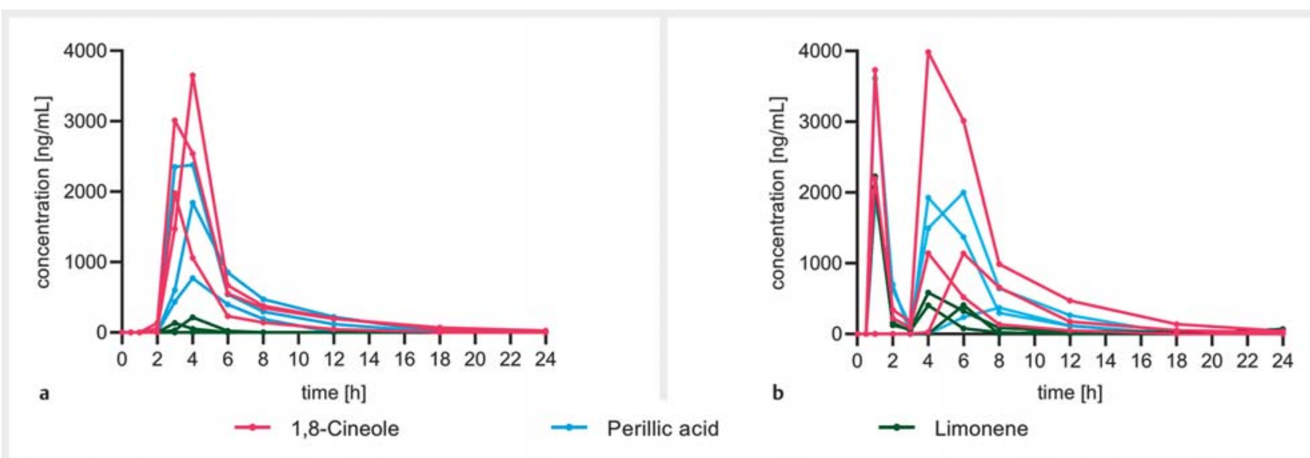
Overlays of individual plasma concentration vs. time curves of dogs receiving BNO 1016 under fasting and fed conditions are given in ▶ **Fig. 1 a, b** and of dogs receiving ELOM-080 in ▶ **Fig. 2 a, b**. The corresponding mean curves are depicted in ▶ **Figs. 3** and **4** and the pharmacokinetic evaluation is given in ▶ **Table 1**.

In general, the three analytes determined for BNO 1016 demonstrated a similar course in the fasted as well as in the fed state by the visual impression. Typical characteristics of an immediate release drug formulation with a rapid increase until C_{max} with median t_{max} values of 1 h in the fasted and 2 h in the fed state could be observed for all three analytes detected. Curves showed the typical sigmoidal course after single dose administration (▶ **Fig. 1**). There was no lag time under fasting and only an expected slight delay of 0.5 h under fed conditions. The rate and extent of exposure [C_{max}, $AUC_{(0\text{-tlast})}$] of verbenalin decreased after the intake of food (ratio of geometric means fed/fasted: 0.48 and 0.69, respectively), whereas that of the further marker analyte was nearly unchanged (ratio fed/fasted: 0.75 and 1.04, respectively). Geometric mean ratios of C_{max} and $AUC_{(0\text{-tlast})}$ values of gentiopicroside were slightly increased with 1.13 and 1.33, respectively. Mean apparent elimination half-life times of the three analytes lay in the range of 0.96–1.78 h (fasted) and 1.30–2.05 h (fed), suggesting that elimination kinetics are unchanged (see ▶ **Table 1**). Overall, a relevant food effect could not be observed after administration in dogs.

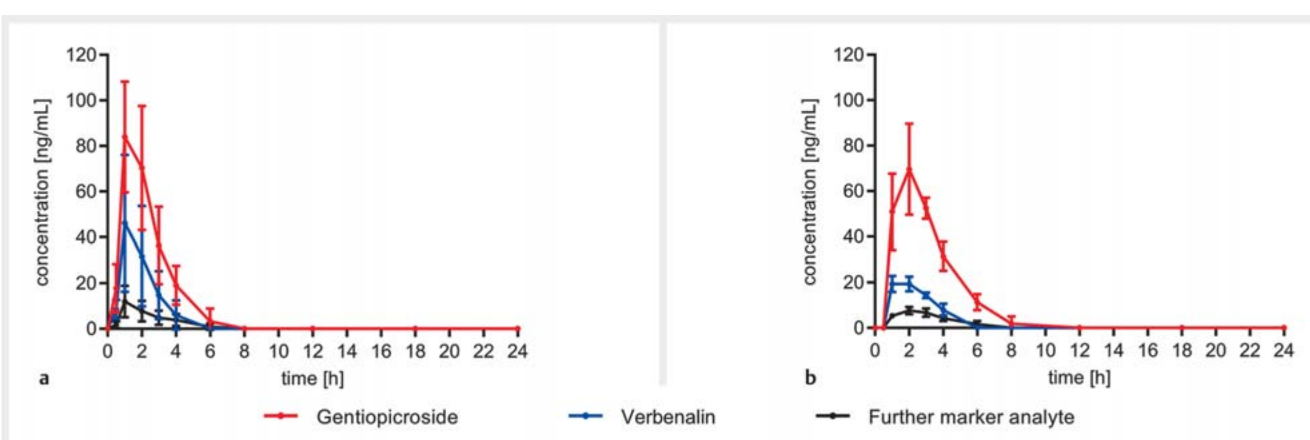
Individual overlays of the ELOM-080 analytes in the fasted state also showed an overall similar picture in the three dogs (▶ **Fig. 2**). Interestingly, lag times of up to 2 h p. a. could be observed for at least two of the three analytes, reflecting a longer residence time of the formulations in the dogs' stomach until drug liberation in the intestine. Corresponding t_{max} values lay in the range of 3 to 4 h p. a. The individual overlays after the administration of the high-fat diet show two major peaks. This occurred in two of the three animals. A sigmoidal course after administration can, at best, be guessed for the second major peak of the individual curves. Observed median t_{lag} was 0.5 h but from 0.5 to 4 h for all analytes. Raw data of one of the three dogs revealed only 1 peak for all analytes with t_{max} values up to 8 h and t_{lag} of 4 h. C_{max} and $AUC_{(0\text{-tlast})}$ of 1,8-cineole (ratio of geometric means fed/fasted: 0.92 and 1.12, respectively) and perillic acid (ratio of geometric means fed/fasted: 0.92 and 0.90, respectively) were in the same magnitude under fasted and fed administration, whereas that of limonene was increased (ratio fed/fasted: 5.77 and 23.36, respectively). Mean apparent elimination half-life times of the analytes



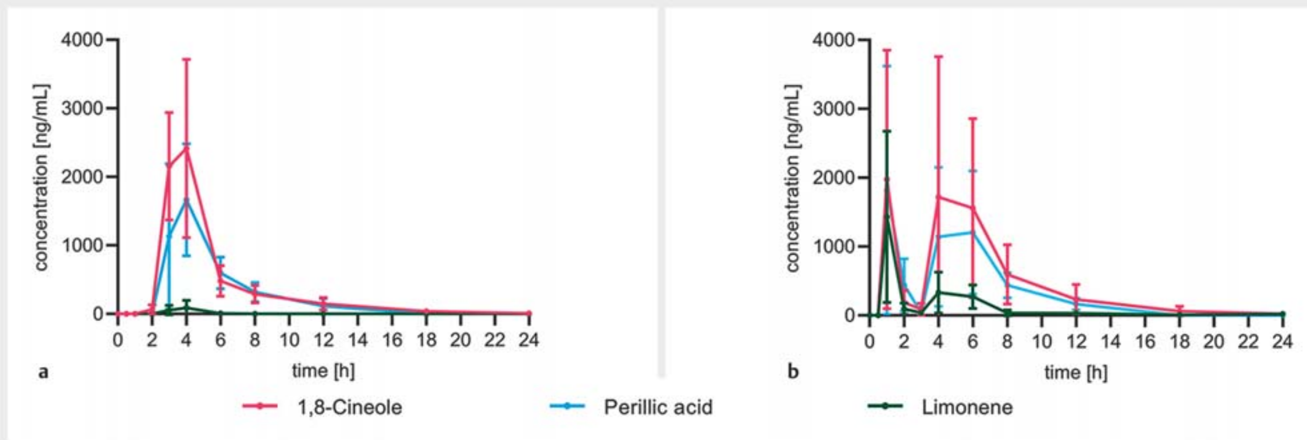
► **Fig. 1** Overlay of individual plasma concentration vs. time curves of gentiopicroside, verbenalin, and a further marker analyte in dogs (n = 3) after administration of BNO 1016 under fasting (**a**) and fed (**b**) conditions. For graphical presentation, all concentrations of BLOQ are set to "0".



► **Fig. 2** Overlay of individual plasma concentration vs. time curves of 1,8-cineole, perillic acid, and limonene in dogs (n = 3) after administration of ELOM-080 under fasting (**a**) and fed (**b**) conditions. For graphical presentation, all concentrations of BLOQ are set to "0".



► **Fig. 3** Mean plasma concentration vs. time curves (± SD) of gentiopicroside, verbenalin, and a further marker analyte in dogs (n = 3) after administration of BNO 1016 under fasting (**a**) and fed (**b**) conditions. For graphical presentation, all concentrations of BLOQ are set to "0".



▸ **Fig. 4** Mean plasma concentration vs. time curves (± SD) of 1,8-cineole, perillic acid, and limonene in dogs (n = 3) after administration of ELOM-080 under fasting (**a**) and fed (**b**) conditions. For graphical presentation, all concentrations of BLOQ are set to "0".

▸ **Table 1** Pharmacokinetic parameters after administration of ELOM-080 and BNO 1016 in the fasted and fed states.

		ELOM-080			**BNO 1016**		
Fasted state		**1,8-Cineole**	**Limonene**	**Perillic acid**	**Gentio-picroside**	**Verbenalin**	**Further marker analyte**
N		3	3	3	3	3	3
$AUC_{(0\text{-tlast})}$	Geom. mean (CV%) [hng/mL]	7996.87 (52.11)	123.77 (194.23)	8329.68 (19.98)	160.44 (39.98)	75.91 (69.34)	23.51 (74.58)
C_{max}	Geom. mean (CV%) [ng/mL]	2791.06 (32.08)	213.90 (357.61)	1500.42 (64.69)	65.84 (23.13)	40.69 (65.07)	10.77 (60.15)
t_{max}	Median (range) [h]	3 (3–4)	4 (3–6)	4 (4–4)	1 (1–1)	1 (1–1)	1 (1–1)
t_{lag}	Median (range) [h]	1.02 (1.02–2)	2 (2–2)	2 (2–2)	0 (0–0)	0 (0–0)	0 (0–0.5)
$t_{1/2}$	Mean (SD) [h]	3.51 (1.25)	–	2.98 (0.28)	1.09 (0.11)	0.96 (3.34)	1.78 (0.43)
Fed state		**1,8-Cineole**	**Limonene**	**Perillic acid**	**Gentio-picroside**	**Verbenalin**	**Further marker analyte**
N		3	3	3	3	3	3
$AUC_{(0\text{-tlast})}$	Geom. mean (CV%) [hng/mL]	8963.18 (82.49)	2890.91 (45.14)	7478.24 (190.63)	213.18 (7.88)	52.08 (7.71)	24.47 (33.29)
C_{max}	Geom. mean (CV%) [ng/mL]	2566.48 (80.36)	1234.14 (123.21)	1373.78 (171.89)	74.62 (29.61)	19.38 (18.45)	8.07 (21.10)
t_{max}	Median (range) [h]	4 (1–6)	1 (1–6)	4 (1–8)	2 (2–3)	2 (1–2)	2 (2–3)
t_{lag}	Median (range) [h]	0.5 (0.5–3)	0.5 (0.5–4)	0.5 (0.5–4)	0.5 (0.5–0.5)	0.5 (0.5–0.5)	0.5 (0.5–0.5)
$t_{1/2}$	Mean (SD) [h]	3.09 (1.11)	–	1.97 (0.26)	1.30 (0.23)	2.05 (1.574)	2.05 (0.306)
Ratio fed/fasted	$AUC_{(0\text{-tlast})}$	1.12	23.36	0.90	1.33	0.69	1.04
	C_{max}	0.92	5.77	0.92	1.13	0.48	0.75

lay in the range from 2.98 to 3.51 h (fasted) and 1.97 to 3.09 h (fed). Although $t_{1/2}$ of limonene could not be derived properly, the data suggest that elimination kinetics are not altered by food intake, as expected (see ▸ **Table 1**).

Discussion

The present pilot study investigated the pharmacokinetics of BNO 1016 and ELOM-080 with special attention to (1) the absorption and (2) the occurrence of a food effect depending on preceding feed intake in male beagle dogs. This was an exploratory pilot study and therefore a sample size calculation for a confirmatory trial did not take place. We chose three Beagle dogs as from a 3R perspective: the use of larger sample sizes would not be ethically acceptable, i.e., if results can be obtained from smaller sample sizes. Both herbal medicinal products are widely used in Germany and Europe for the treatment of acute rhinosinusitis. BNO 1016 is available as an immediate release formulation (coated tablet) and ELOM-080 is contained in an enteric-coated soft gelatin capsule. It can be assumed that absorption of APIs from BNO 1016 occurs fast and therefore, results also in a fast onset of treatment effects. The purpose of an enteric coating is to delay dissolution and the release of APIs from the drug formulation until it has reached the intestine. Therefore, it might be suggested that the retention time of an enteric-coated soft gelatin capsule in the stomach is influenced when food has been consumed prior to ingestion or immediately afterwards. The time indigestible solid particles are retained in the stomach usually depends on the diameter of the dosage form and the frequency of food intake, as well as on the composition and caloric density of an administered meal [20, 21]. Consequently, food effects most frequently manifest themselves in a relevant *in vivo* change in liberation from the dosage form associated with a change in the onset of absorption (t_{lag}), i.e., in pharmacokinetic parameters that are strongly formulation dependent. Furthermore, the extent and rate of bioavailability can be influenced ($AUC_{0\text{-tlast}}$, C_{max}, t_{max}). Conceivable, but less probable, is the influence on distribution and elimination. However, in the case of sudden large increases in dosage, e.g., in the case of a dose-dumping phenomenon [22], changes due to saturation phenomena of eliminating enzymes and transporters are also possible. As a consequence, the EMA guidelines on the pharmacokinetic and clinical evaluation of modified release dosage forms [23] also require the verification of unexpected release characteristics. In humans, published data show that single unit enteric-coated dosage forms may remain in the stomach for 10 h or even longer when coadministered with a high-fat breakfast followed by several meals over the course of the day [24]. Moreover, it is known that gastric emptying of drugs may be even delayed for up to 30 h in non-fasted animals [25].

In this first pilot study, we chose beagle dogs as the standard model to study food effects [18]. Based on our previous experience with the analytical detectability of analytes in plasma from rats and dogs, we chose to administer the 5-fold equivalent of the recommended human daily dose assuming that the physiological performance of the single formulations will not change with the dosage administered. Plasma samples were withdrawn until 30 h p. a. to avoid missing possible late-occurring absorption processes. In general, the analytes determined for BNO 1016 demonstrated a similar course of the individual plasma concentrations vs. time curves in the fasted as well as in the fed state by the visual impression. Lag times of the absorption process lay in the expected range of 0–0.5 h p. a. for an immediate release formulation with corresponding median t_{max} values of 1–2 h. A relevant food effect could be ruled out. For the enteric-coated formulation of ELOM-080, however, we observed lag times of the absorption up to 2 h p. a. even in the fasted state, reflecting longer gastric residence times of the formulations in the dogs. In the fed state, a pronounced food effect could be noticed, with median lag times up to 3–4 h p. a. and a splitting into two major peaks in plasma curves in two of the three dogs. The cause of this double peak phenomenon is unknown. Neither the inspection of the laboratory journals nor the direct questioning of the staff gave any indications of misadministration, e.g., dogs biting capsules etc.

While according to the respective SmPC [4], the intake of ELOM-080 is recommended before meals, a truly empty stomach in humans can usually only be expected in the mornings after overnight fasting. The consumption of meals and snacks throughout the day might practically lead to a closed pylorus sphincter over long time distances during the day [26]. A respective food effect in humans for ELOM-080 should not lead to severe safety-relevant side effects. However, if this finding in dogs translates to humans, some of the frequently observed gastrointestinal side effects, like stomach and upper abdominal complaints [4], might be explained. Most certainly, a precise dosing with a rapid release and onset of action does not seem likely based on the data obtained in the present study. Therefore, it seems quite valuable to verify these findings in a further pharmacokinetic study in humans, considering that patients suffering from respiratory tract disorders take ELOM-080 with the expectation of a fast relief of their complaints.

In conclusion, our pilot study provides evidence that, in contrast to the immediate release medicine BNO 1016 with fast and similar absorption characteristics, absorption of ELOM-080 from an enteric-coated capsule might be substantially affected by food intake in humans. Consequently, a faster and more precise onset of action in humans can be assumed for BNO 1016.

Materials and Methods

Test items and dose selection

For the experiments, commercially available Sinupret extract coated tablets (Batch No.: 0 000 144 647) and Gelomyrtol forte soft gelatin capsules (Batch No.: 260 248) containing BNO 1016 or ELOM-080, respectively, were purchased in a public pharmacy.

A coated tablet of Sinupret extract contains 160.00 mg dry extract (3–6 : 1) of gentian root, cowslip flowers, sorrel herb, elderflower, and verbena (1 : 3 : 3 : 3 : 3); extraction solvent: ethanol 51 % (m/m). One enteric-coated soft gelatine capsule of Gelomyrtol forte contains 300 mg distillate from a mixture of rectified eucalyptus oil, rectified sweet orange oil, sweet orange oil, rectified myrtle oil, and rectified lemon oil (66 : 32 : 1 : 1). Previous experiments on BNO1016 showed that the single equivalent human dose is not sufficient for the reliable bioanalytical detection of the lower concentrated analytes. Therefore, doses were chosen as high as necessary and as low as possible and the dosage of ELOM-80 was adapted accordingly.

The 5-fold equivalent of the recommended human daily dose was chosen [BNO 1016: 5 tablets per animal, i.e., 72 (± 2.2) mg/kg, ELOM-080: 6 capsules per animal, i.e., 162 (± 5.0) mg/kg] to ensure bioanalytical detectability of the selected analytes in plasma.

Study design and animal treatment

The study protocol was approved by the Avogadro LS Animal Ethics Committee on July 7, 2020, according to the reference number approval 2017021409405658 (id 062) authorized by the French Ministry for higher education and research. Animal housing and care complied with the recommendations of Directive 2010/63/EU.

Three non-naïve male adult beagle dogs [mean age 42 (± 8.7) months; Marshall BioResources] weighing 10.9 to 11.4 kg, collectively housed in pens, were used in the study, which was performed according to a 4-period, 4-treatment within-design. Animals received all treatments under both fasted (periods 1 and 3) and fed (periods 2 and 4) conditions. They were fasted overnight in each period for at least 8 h prior to dosing. In periods 1 and 3, a high-fat diet was given about 3 h after administration (fasted state).

The high-fat diet consisted of the following ingredients: 2 large eggs, 50 g of bacon, 130 g of bread, 15 g of sunflower oil, 15 g of butter, and 190 g of tap water. The ingredients were homogenized in a laboratory blender. Diet was prepared and then stored in 50 g aliquots at ca. − 20 °C and used within 1 month maximum. Aliquots were defrosted overnight at ca. + 5 °C before use. Composition of the high-fat diet was as follows: 17% fat, 11% protein, and 18% carbohydrate (on a fresh matter basis). In periods 2 and 4, the same high-fat diet was given approximately 10 min prior to dosing. Then, regular feeding (Teklad 2027, Envigo Teklad Diets) was given about 12 h post-drug administration (p. a.) in each period. Water was offered ad libitum. Treatments were separated for at least 7 treatment-free days to guarantee a sufficient washout of the drugs.

Test items were administered orally directly into the back of the throat of the animals. Ten mL of water wer added directly into the mouth during administration to ensure good esophageal transit and then 50 mL was administered by gavage using a gastroesophageal tube to mimic ingestion with liquid in humans as recommended.

Blood sampling

Blood samples (2.5 mL) were withdrawn into lithium heparin tubes from the jugular vein. Samples were collected at the following time points: pre-dose and 0.5, 1, 2, 3, 4, 6, 8, 12, 18, 24, 26, 28, and 30 h p. a. Real blood sampling times were noted.

Bioanalytics and pharmacokinetics

After withdrawal, blood samples were immediately centrifuged at 2500 × *g* for 10 min at + 5 °C and plasma obtained was cooled in dry ice. Samples were set on dry ice and stored at ≤ − 70 °C until measurement. A maximum period of 8 weeks elapsed from blood withdrawal to measurement. Reliably detectable and representative ingredients or metabolites were selected for measurement: 1,8-cineole, limonene, and its metabolite perillic acid were determined for ELOM-80, and gentiopicroside, verbenalin, and a further marker analyte for BNO 1016. This further analyte is considered a standard marker substance for BNO 1016 but shall not be described in more detail in here for internal company reasons.

The monoterpene metabolite perillic acid as well as gentiopicroside, verbenalin, and the further marker analyte were determined after protein precipitation by LC-MS/MS. For sample preparation, 50 µL plasma samples were mixed with internal standard solution (200 ng/mL methylparaben (for perillic acid), 30 ng/mL amarogentin (for gentiopicroside, verbenalin), 40 ng/mL eriocitrin (for the further marker analyte) in 1% formic acid in acetonitrile in a 96-well filter plate (Ostro Protein Precipitation & Phospholipid Removal Plate, Waters). The filter plate was placed in the test tube shaker for 5 min at 800 rpm (pulsating) and subsequently centrifuged for 5 min at 2000 × *g*.

Chromatographic separation was performed on a UHPLC system (1290 Infinity series, Agilent). For the determination of gentiopicroside, verbenalin, and the further marker analyte, a Phenomenex Kinetex C18 column (50 × 2.1 mm, 1.7 µm) was used. The injection volume was 1 µL and the flow rate was set to 0.7 mL/min. For the chromatographic separation, a gradient was run using 0.5% acetic acid in water (solvent A) and acetonitrile (solvent B). For the determination of perillic acid, a Zorbax RRHD Eclipse C18 column (50 × 2.1 mm, 1.8 µm; Agilent) was used. The injection volume was 2 µL and the flow rate was set to 0.6 mL/min. For the chromatographic separation, a gradient was run using 0.1% formic acid in water (solvent A) and acetonitrile (solvent B). Detection was performed using a triple quadrupole mass spectrometer (Triple Quad 6500 or API 4000, Sciex) in MRM mode. Measurements were carried out in the positive electrospray ionization mode. The system was controlled using Analyst 1.7.1 software (Sciex).

For monoterpene analysis, 300 µL plasma samples were processed by solid-phase extraction. 1,8-Cineole and limonene were quantitatively determined by GC-MS. For sample preparation, plasma samples were added to the internal standard solution (2.0 µg/mL menthol in methanol) in Eppendorf tubes. Afterwards, SPE cartridges (C18 cartridge; Waters) were equilibrated with methanol, then with water. Samples mixed with the internal standard were loaded onto the cartridge. The eluate was collected and loaded onto the cartridge twice more, then the cartridge was washed three times with water. Finally, the analytes were eluted with a mixture of ethylacetate/hexane. A sample vial was filled with the upper phase and used for the GC-MS measurement. The separation was carried out on a gas chromatograph 7890A from Agilent using an Agilent HP-Innowax capillary column (30 m × 320 µm × 0.25 µm). The injection volume was 1.5 µL. A temperature gradient of the column oven was run. Detection was performed using a mass spectrometer (5975C, Agilent with electron impact ionization in selected ion monitoring mode).

Limonene (purity 99.6% by GC), perillic acid (purity 99.4% by titration), menthol (purity ≥ 98% by GC), and methylparaben (content > 99%) were purchased from Sigma-Aldrich, eriocitrin (content 88.8%) was from HWI Analytik GmbH, and amarogentin (content 91%), 1,8-cineole (content 99%), gentiopicroside (content 96%), and verbenalin (content 100%) werefrom PhytoLab.

Calibration ranges were 20–10 000 ng/mL for 1,8-cineole and 20–5000 ng/mL for limonene. Perillic acid had a range of 100–9000 ng/mL, gentiopicroside and verbenalin a range of 5–1000 ng/mL, and the further marker analyte had a range of 2–400 ng/mL. The lowest concentration indicated for each concentration range represents the respective LLOQ. Calibration ranges covered the plasma concentrations of all study samples. Calibration and quality control samples were prepared in canine plasma. The analytical method used for the quantification of gentiopicroside, verbenalin, and the further marker analyte was fully validated in compliance with relevant EMA guidelines for bioanalytical method validation [27]. The performance of all analytical methods used was controlled via QC samples.

For the LC-MS/MS methods, correlation coefficients of 0.9996 (perillic acid, verbenalin), 0.9997 (further marker analyte), and 0.9998 (gentiopicroside) were obtained. Accuracies of the calibration samples were in the range of 93.17–104.8% (perillic acid), 93.94–105.2% (verbenalin), 94.82–107.1% (gentiopicroside), and 94.18–112.3% (further marker analyte). QC sample accuracies ranged between 83.43–95.79% (perillic acid), 93.08–109.9% (gentiopicroside), 91.94–108.6% (verbenalin), and 88.89–115.0% (further marker analyte).

For the GC-MS methods, correlation coefficients ranged between 0.9996–0.9998 (1,8-cineole) and 0.9981–0.9997 (limonene). For the calibration samples, accuracies were in the range of 91.26–117.8% (1,8-cineole) and 89.35–116.5% (limonene). QC sample accuracies ranged between 84.95–109.3% (1,8-cineole) and 83.01–143.8% (limonene).

Pharmacokinetic parameters derived were determined model independently by non-compartmental analysis using Certara Phoenix 64 WinNonlin software (version 8.2.0.4383). Plasma concentrations below LLOQ were set to "0" before C_{max} and afterwards omitted. C_{max} and t_{max} values were read directly from the observed concentration-time points. Areas under the curves were calculated according to the linear/log trapezoidal rule, which uses linear data obtained during the absorption phase up to C_{max} and logarithmically transformed concentrations thereafter. Although not the focus of this work, the apparent terminal elimination half-life was calculated using nonlinear regression on data points visually assessed to be on the terminal phase of concentration curves. Half-life times were only determined when meaningful, i.e., when at least three measured values above LLOQ were available in the elimination phase. The lag time was taken as the time interval from dosing to the sampling time point for the first quantifiable concentration of an analyte. For graphical presentations, all plasma concentrations below LLOQ were set to "0".

Contributors' Statement

Design of the study: J. Seibel, M. Wonnemann. Bioanalytics: A. Neumann, A. Müller. Pharmacokinetics: M. Wonnemann. Analysis and interpretation of data: S. Seibel, A. Neumann, A. Müller, M. Wonnemann. Medical writing: M. Wonnemann, J. Seibel.

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Conflict of Interest

Financial: Jan Seibel and Meinolf Wonnemann are employees of Bionorica SE, Germany. Financial: Astrid Neumann and Anne Müller are employees of Bionorica research GmbH, Austria.

References

[1] Mäder K. Innovative Arzneiformen: Ein Lehrbuch für Studium und Praxis [Innovative Dosage Forms: A Textbook for Study and Practice], 1st edition. Stuttgart: Wissenschaftliche Verlagsgesellschaft Stuttgart; 2009

[2] Uhl P. Schnell oder langsam, wie sich die Freisetzung von Arzneimitteln modifizieren lässt [Fast or slow, how to modify the release of drugs]. DAZ 2019; 28: 44

[3] Bayrischer Apothekerverband. Marktdaten Selbstmedikation: Gesamtjahr 2020 [Self-medication Market Data: Full Year 2020]. OTC Manager 2021; 3: 8–33

[4] G. Pohl-Boskamp GmbH & Co. KG. Summary of product characteristics: Gelomyrtol forte. Approval No.: 6096046.00.00, Feb 2018. Accessed February 17, 2022 at: https://www.fachinfo.de/suche/fi/007773

[5] Han D, Wang N, Zhang L. The effect of myrtol standardized on human nasal ciliary beat frequency and mucociliary transport time. Am J Rhinol Allergy 2009; 23: 610–614

[6] Dorow P, Weiss T, Felix R, Schmutzler H. Effect of a secretolytic and a combination of pinene, limonene and cineole on mucociliary clearance in patients with chronic obstructive pulmonary disease. Arzneimittelforsch 1987; 37: 1378–1381

[7] Behrbohm H, Kaschke O, Sydow K. Effect of the phytogenic secretolytic drug Gelomyrtol forte on mucociliary clearance of the maxillary sinus. Laryngorhinootologie 1995; 74: 733–737

[8] Zhang S, Skinner D, Hicks SB, Bevensee MO, Sorscher EJ, Lazrak A, Matalon S, McNicholas CM, Woodworth BA. Sinupret activates CFTR and TMEM16A-dependent transepithelial chloride transport and improves indicators of mucociliary clearance. PLoS One 2014; 9: e104090

[9] Kreindler JL, Chen B, Kreitman Y, Kofonow J, Adams KM, Cohen NA. The novel dry extract BNO 1011 stimulates chloride transport and ciliary beat frequency in human respiratory epithelial cultures. Am J Rhinol Allergy 2012; 26: 439–443

[10] Virgin F, Zhang S, Schuster D, Azbell C, Fortenberry J, Sorscher EJ, Woodworth BA. The bioflavonoid compound, Sinupret, stimulates transepithelial chloride transport *in vitro* and *in vivo*. Laryngoscope 2010; 120: 1051–1056

[11] Bionorica SE. Summary of product characteristics: Sinupret extract. Approval No.: 84060.00.00, Jan 2018. Accessed February 17, 2022 at: https://www.fachinfo.de/suche/fi/014213

[12] Rossi A, Dehm F, Kiesselbach C, Haunschild J, Sautebin L, Werz O. The novel Sinupret dry extract exhibits anti-inflammatory effectiveness *in vivo*. Fitoterapia 2012; 83: 715–720

[13] Karim A, Burns T, Wearly L, Streicher J, Palmer M. Food-induced changes in theophylline absorption. from controlled-release formulations. Part I. Substantial increased and decreased absorption with Uniphyl tablets and Theo-Dur Sprinkle. Clin Pharmacol Ther 1985; 38: 77–83

[14] Karim A, Burns T, Janky D, Hurwitz A. Food-induced changes in theophylline absorption from controlled-release formulations. Part II. Importance of meal composition and dosing time relative to meal intake in assessing changes in absorption. Clin Pharmacol Ther 1985; 38: 642–647

[15] Waldman SA, Morganroth J. Effect of food on the bioequivalence of different verapamil sustained-release formulations. J Clin Pharmacol 1995; 35: 163–169

[16] Blume HH, Schug BS, Potthast H. Influence of food on the bioavailability of controlled/modified release products. In: Kuhlmann J, Weihrauch TR, eds. Clinical pharmacology 12, food–drug interactions. München, Bern, Wien, New York: Zuckschwerdt; 1996: 25–32

[17] Szurszewski JH. A migrating electric complex of the canine small intestine. Am J Physiol 1969; 217: 1757–1763

[18] Sjögren E, Abrahamsson B, Augustijns P, Becker D, Bolger MB, Brewster M, Brouwers J, Flanagan T, Harwood M, Heinen C, Holm R, Juretschke HP, Kubbinga M, Lindahl A, Lukacova V, Münster U, Neuhoff S, Nguyen MA, Peer Av, Reppas C, Hodjegan AR, Tannergren C, Weitschies W, Wilson C,

Zane P, Lennernäs H, Langguth P. *In vivo* methods for drug absorption – comparative physiologies, model selection, correlations with *in vitro* methods (IVIVC), and applications for formulation/API/excipient characterization including food effects. Eur J Pharm Sci 2014; 57: 99–151

[19] Lentz KA. Current methods for predicting human food effect. AAPS J 2008; 10: 282–288

[20] Ewe K, Press AG, Bollen S, Schuhn I. Gastric emptying of indigestible tablets in relation to composition and time of ingestion of meals studied by metal detector. Digest Dis Sci 1991; 36: 146–152

[21] Davis SS, Hardy JG, Fara JW. The transit of pharmaceutical dosage forms through the small intestine. Gut 1986; 27: 886–892

[22] Hendeles L, Weinberger M, Milavetz G, Hill M 3rd, Vaughan L. Food-induced “dose-dumping” from a once-a-day theophylline product as a cause of theophylline toxicity. Chest 1985; 87: 758–765

[23] European Medicines Agency (EMA): EMEA/CHMP/EWP/280/96 Rev1. Guideline on the pharmacokinetic and clinical evaluation of modified release dosage forms. November 2014. Accessed February 17, 2022 at: https://www.ema.europa.eu/en/pharmacokinetic-clinical-evaluation-modified-release-dosage-forms

[24] Schug BS, Brendel E, Chantraine E, Wolf D, Martin W, Schall R, Blume HH. The effect of food on the pharmacokinetics of nifedipine in two slow release formulations: pronounced lag-time after a high fat breakfast. J Clin Pharmacol 2002; 53: 582–588

[25] Aoyagi N, Ogata H, Kaniwa N, Uchiyama M, Yasuda Y, Tanioka Y. Gastric emptying of tablets and granules in humans, dogs, pigs, and stomach-emptying-controlled rabbits. J Pharm Sci 1992; 81: 1170–1174

[26] Ramkumar D, Schulze KS. The pylorus. Neurogastroenterol Motil 2005; 17: 22–30

[27] European Medicines Agency (EMA): EMEA/CHMP/EWP/192217/2009. Guideline on bioanalytical method validation. February 2012. Accessed February 17, 2022 at: https://www.ema.europa.eu/en/bioanalytical-method-validation