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# Analysis of erythromycin and benzoylperoxide in topical gels by liquid chromatography

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**Abstract**

Gels containing a combination of erythromycin and benzoylperoxide are frequently used in the treatment of acne vulgaris. A method was developed to determine the content of both erythromycin and benzoylperoxide in these gels. Erythromycin was extracted from the gel in conditions where the oxidative power of benzoylperoxide was neutralised by addition of ascorbic acid and this extract was analysed on an Xterra $RP_{18}$ column, with a mobile phase containing acetonitrile–0.2 *M* $K_2HPO_4$–water (35:5:60, v/v/v). The detection wavelength was 215 nm. A second extraction procedure was developed for the analysis of benzoylperoxide. The extraction solution was analysed on a Hypersil $C_{18}$ BDS column and a mobile phase containing acetonitrile–water (58:42, v/v). Detection was performed at 254 nm. The flow rate was 1.0 ml/min in both methods. The selectivity, repeatability, linearity and recovery of both methods were examined. Special attention was given to determination of the recovery and the uncertainty on the recovery. This allowed evaluation of the bias of the extraction method. The method developed was used to examine the stability of a gel for topical use.
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*Keywords:* Erythromycin; Benzoylperoxide

## 1. Introduction

Gels containing a combination of erythromycin and benzoylperoxide are frequently used in the treatment of acne vulgaris. Erythromycin is a macrolide antibiotic produced by *Saccharopolyspora erythreas* during fermentation. The main component of erythromycin is erythromycin A (EA) [1]. The chemical structure of EA is shown in Fig. 1. Erythromycin is used in the treatment of acne because of its bacteriostatic activity against *Propionibacterium acnes*, which can be found in the sebum [2–4]. Benzoylperoxide acts as a keratolyticum and also has antibacterial activity against *Propionibacterium acnes* because of its oxidative power [5]. The chemical structure of benzoylperoxide is shown in Fig. 2.

To assay the content of both substances and to follow their stability in a gel, it is important to have

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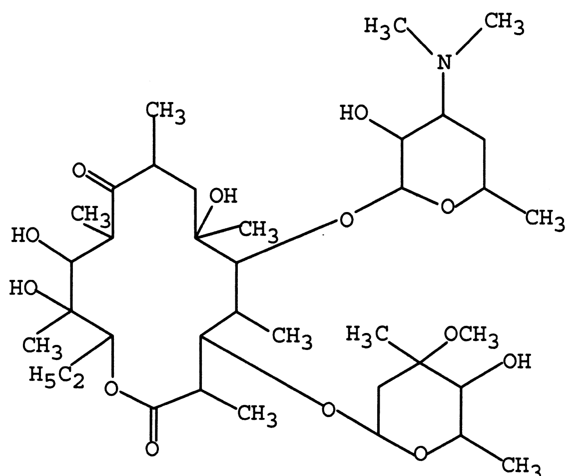


Fig. 1. Chemical structure of erythromycin A.

a good analytical method. Only one method for the analysis of both active substances in gels has been published [6]. In this method benzoylperoxide was assayed by liquid chromatography (LC), while erythromycin was determined with a microbiological method. The aim of this work was to develop LC methods for both compounds. The assay of erythromycin by LC offers several advantages, such as high specificity, the possibility of determining and quantifying impurities and degradation products, and improved accuracy. Solutions containing both erythromycin and benzoylperoxide are not stable because erythromycin is readily oxidized and derivatised by benzoylperoxide. Therefore sample preparation is more difficult than usual.

The developed methods were validated. Repeatability of the extraction procedures, linearity and recovery were examined. Special attention was given to determination of the recovery and to its uncertainty. Recovery can be defined as "the ratio of the observed result for the method to a reference value" [7] or as "the proportion of amount of analyte, present or added to the analytical portion of the test

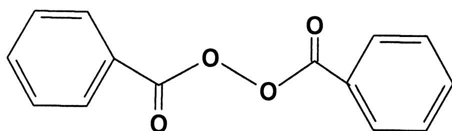


Fig. 2. Chemical structure of benzoylperoxide.

material, which is extracted and presented for measurement" [8]. Recovery assays can be used for trueness assessment [9]. In this recovery study the bias of the procedure will be estimated. If a bias exists, the recovery is statistically different from 1 and a correction for recovery can be taken into account.

After the methods were validated, they were used to examine the stability of erythromycin and benzoylperoxide in topical gels.

## 2. Materials and methods

### 2.1. Reagents and samples

Acetonitrile, HPLC grade "S" was from Biosolve (Valkenswaard, The Netherlands). Dipotassium hydrogen phosphate (Merck, Darmstadt, Germany) 0.2 *M* solution was adjusted to the required pH by addition of 0.2 *M* phosphoric acid (Merck). Ascorbic acid was from Acros Organics (Geel, Belgium) and potassium hydroxide (KOH) was obtained from Riedel-de Haën (Seelze, Germany). The content of a commercial sample of benzoylperoxide (Merck, Darmstadt, Germany) was determined by iodometric titration [7]. For safety reasons (explosion) benzoylperoxide is commercialized as a hydrous substance, containing ~75% m/m in water. This sample was used as reference substance in the determination of the benzoylperoxide content. The erythromycin starting material used in the preparation of the gel was considered to represent 100% and was used as a reference substance in the determination of erythromycin. Demineralised water was distilled with glass apparatus. The samples (Benzamycin®, Trenker, Belgium) contained 3% (m/m) of erythromycin and 5% (m/m) of anhydrous benzoylperoxide. Other components of the gel were carbomer 940 or carbomer 980 (Benzamycin 940, Benzamycin 980), sodium hydroxide, ethanol 90°, lemon oil, methylsalicylate, sodium dodecyl sulfosuccinate and purified water. The drug product is commercialized as three separate components: erythromycin powder, ethanol (3.0 ml) and the gel containing all the other ingredients. Because of stability reasons, erythromycin, in 10% overdose, has to be added to the Benzamycin gel immediately before delivery by the

pharmacist. After dissolution in 3.0 ml of ethanol, 800 mg of erythromycin is mixed with 23.3 g of gel containing all the other ingredients. This gel is labeled to be stable for 2 months if stored in a refrigerator (2–8 °C). In the recovery study, two different matrices were used: Benzamycin 940 and a carbomer gel containing carbomer, sodium ethylene diamine tetraacetate, propylene glycol, trometamol and water (Pannoc Chemie, Olen, Belgium). The second matrix was examined in order to verify whether the methods, developed to analyse Benzamycin, are suitable for the analysis of similar in-house preparations as well.

### *2.2. Instrumentation and chromatographic conditions*

LC of erythromycin was performed with a SpectraSystem P1000XR quaternary pump, a Spectraseries AS100 autosampler with a 100-μl loop, and a Spectra 100 variable-wavelength UV–Vis detector (all from Thermo Separation Products, Fremont, CA, USA). Data were recorded with a Hewlet-Packard (Avondale, PA, USA) model HP 3396 series II integrator. As stationary phase an Xterra $RP_{18}$ column, 250×4.6 mm I.D., 5 μm (Waters, Milford, MA, USA) was used. The column was kept at 65 °C in a water bath. Isocratic separation was achieved with a mobile phase consisting of acetonitrile–0.2 *M* phosphate buffer, pH 7.0–water (35:5:60, v/v/v), at a flow rate of 1.0 ml/min. The 0.2 *M* phosphate buffer, pH 7.0 was prepared by mixing 0.2 *M* dipotassium phosphate and 0.2 *M* phosphoric acid. The detection wavelength was 215 nm.

LC of benzoylperoxide was performed with a SpectraSystem P1000XR quaternary pump, a Spectraseries AS100 autosampler with a 100-μl loop (all from Thermo Separation Products), and a L-4000 UV detector (Merck Hitachi, Darmstadt, Germany). Data were recorded with a Hewlet-Packard model HP 3396 series II integrator. The stationary phase consisted of a Hypersil BDS $C_{18}$ analytical column (250×4.6 mm I.D.), 5 μm (Alltech, Lokeren, Belgium), kept at 35 °C in a water bath. A mobile phase of acetonitrile–water (58:42, v/v) was used at a flow rate of 1.0 ml/min. The detection wavelength was 254 nm. All mobile phases were degassed by purging with helium.

### *2.3. Sample preparation*

#### *2.3.1. Analysis of erythromycin*

To prepare the dissolution mixture 5 g of ascorbic acid was added to 500 ml of the 0.2 *M* potassium phosphate buffer, pH 7.0, used to prepare the mobile phase. This mixture was brought to pH 7.0 with a 45% (m/v) potassium hydroxide solution. Gel (1.000 g) was diluted with dissolution mixture/acetonitrile (75:25, v/v), brought to 100.0 ml and stirred for 30 min. This mixture was filtered through a 0.2-μm membrane filter and 100 μl of the filtrate was injected into the system.

As reference solution, 30.0 mg of erythromycin reference substance (the starting material for the preparation) was dissolved in dissolution mixture/acetonitrile (75:25, v/v) and brought to 100.0 ml. Then 100 μl of this solution was injected into the LC system.

#### *2.3.2. Analysis of benzoylperoxide*

Gel (0.500 g) was diluted with acetonitrile/water (80:20, v/v), brought to 100.0 ml and stirred for 30 min. This mixture was filtered through a 0.2-μm membrane filter. Then 10.0 ml of this filtrate was diluted to 100.0 ml with the same dissolution mixture and 100 μl of this solution was injected.

As reference solution, 25.0 mg of benzoylperoxide reference substance was dissolved in acetonitrile/water (80:20, v/v) and diluted to 100.0 ml. Then 10.0 ml of this solution was diluted to 100.0 ml with the same dissolution mixture and 100 μl of this dilution was injected into the LC system.

### *2.4. Recovery study*

In order to examine whether a correction for recovery had to be applied, a recovery study was performed. The recovery study was carried out with spiked samples, which were representative for the routine samples, at different concentration levels. The experimental design applied was based on Ref. [9] and consisted of a four-factor fully-nested design. Four factors were studied: concentration of analyte added, matrix, day and replication. The set-up is shown in Fig. 3. For each of the three amounts of analyte added ($l = 3$), the recovery was estimated in two different matrices ($p = 2$). For each matrix, the

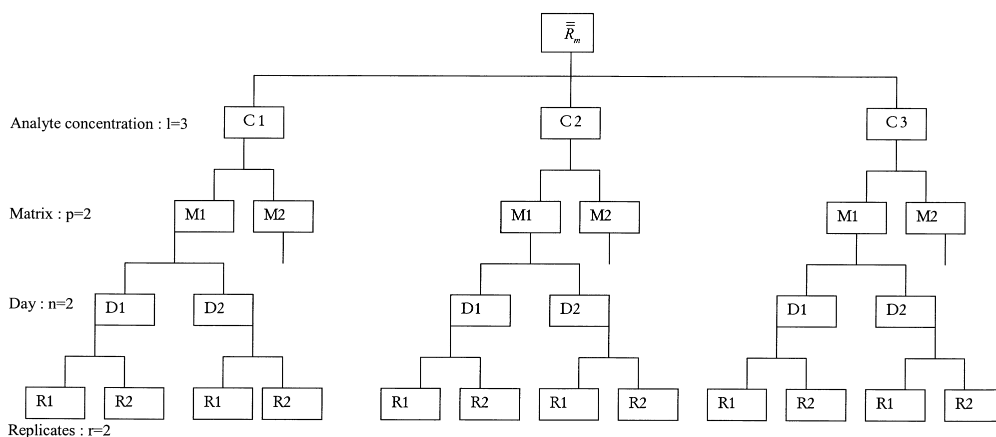


Fig. 3. Set-up of the recovery experiment. $\bar{\bar{R}}_m$, overall recovery; $C$, concentration, $l=3$, number of concentrations; $M$, matrix, $p=2$, number of matrices; $D$, day, $n=2$, number of days; $R$, replicate, $r=2$, number of replicates.

analysis was carried out on two different days ($n=2$), and two replicates were made each day ($r=2$). The replicates consisted of two injections of one extract. In this set-up the variability of the concentration of analyte added can be considered as a fixed effect, while the variabilities of the other factors matrix, day and injection are random effects. The recovery was estimated using the averaged recovery method. The overall recovery, $\bar{\bar{R}}_m$, was calculated by the following equation:

$$\bar{\bar{R}}_m = \frac{\sum_{i=1}^{l} \bar{\bar{R}}_i}{l} \tag{1}$$

where $\bar{\bar{R}}_i$ is the mean recovery for a given amount of analyte added and $l$ is the number of different amounts added.

To check whether the bias is statistically significant, whether the overall recovery is statistically different from 1 is tested. The overall recovery is not statistically different from 1 if:

$$\left|\bar{\bar{R}}_m - 1\right| \leq t_{\alpha/2,\mathrm{eff}}\, u(\bar{\bar{R}}_m) \tag{2}$$

where $u(\bar{\bar{R}}_m)$ is the uncertainty of the overall recovery and $t_{\alpha/2,\mathrm{eff}}$ the two-sided tabulated $t$-value for the effective degrees of freedom associated to $u(\bar{\bar{R}}_m)$. The uncertainty of the overall recovery is calculated as:

$$u(\bar{\bar{R}}_m) = \sqrt{\frac{\sum_{i=1}^{l} u(\bar{\bar{R}}_i)^2}{l}} \tag{3}$$

where $u(\bar{\bar{R}}_i)$ is the uncertainty of each mean recovery. This uncertainty can be estimated in two different ways: (i) as the standard deviation on $\bar{\bar{R}}_i$:

$$u(\bar{\bar{R}}_i) = \sqrt{\frac{\sum_{j=1}^{p} (\bar{R}_{ij} - \bar{\bar{R}}_i)^2}{p-1}} \tag{4}$$

where $\bar{R}_{ij}$ is the average recovery at a given amount added and for a given matrix and $p$ is the number of different matrices, or (ii) using the information of the intermediate precision of the method:

$$u(\bar{\bar{R}}_i)^2 = \frac{s_I^2}{\bar{x}_a^2 npr} \tag{5}$$

where $s_I^2$ is the intermediate precision of the concentration found for a given amount $x_a$ added and $n$, $p$ and $r$ are explained in Fig. 3. As this analytical method had been only recently developed, no historical data providing information about the intermediate precision were available. Therefore the intermediate precision was estimated from the variance analysis of the nested design result. Table 1 shows the equations for the expected mean square (MS) results. From the estimated and the expected MS results, the variances $s^2$ were calculated. These variances are expressed as uncertainty estimates. The repeatability variance,

Table 1
ANOVA table showing the mean squares (MS) at each level

| Source | Levels | MS, erythromycin | MS, benzoylperoxide | df | Expected MS |
|---|---|---|---|---|---|
| Concentration | $l=3$ | $6.997\times10^{-5}$ | $1.032\times10^{-4}$ | 2 | $pnrs^2(C)+nrs^2(M)+rs^2(D)+s^2(i)$ |
| Matrix | $p=2$ | $2.283\times10^{-4}$ | $7.922\times10^{-4}$ | 3 | $nrs^2(M)+rs^2(D)+s^2(i)$ |
| Between-day | $n=2$ | $9.704\times10^{-4}$ | $1.252\times10^{-3}$ | 6 | $rs^2(D)+s^2(i)$ |
| Between-injection | $r=2$ | $1.162\times10^{-4}$ | $1.796\times10^{-4}$ | 12 | $s^2(i)$ |
| Total | | | | 23 | |

$u(r)^2=u(i)^2$, and the variance between days, $u(D)^2$, between different matrices, $u(M)^2$, and between different concentrations, $u(C)^2$, were calculated. The intermediate precision then becomes:

$$s_I^2=u(I)^2=u(r)^2+u(D)^2 \tag{6}$$

When recoveries are determined in the nested design, the precision estimates obtained are relative standard deviations (RSD) instead of standard deviations. Therefore, the intermediate precision obtained in the nested design corresponds to the relative intermediate standard deviation (i.e. $RSD_I=s_I/x_a$). Therefore Eq. (5) can be expressed as:

$$u(\bar{\bar{R}}_i)^2=\frac{u(I)^2}{npr} \tag{7}$$

## 3. Results and discussion

### 3.1. Method development

Because of the reactivity of benzoylperoxide, solutions containing erythromycin and benzoylperoxide are unstable. Two separate extraction procedures were developed and each extract was analysed for one of the active components. Erythromycin was extracted from the gel in conditions where the oxidative power of benzoylperoxide was neutralised by the addition of ascorbic acid. The stability of erythromycin is optimal in the pH 7–8 range. EA degrades in mild acidic conditions to erythromycin A enol ether (EAEN) and anhydroerythromycin A (AEA) [10,11]. At slightly alkaline or alkaline pH, pseudoerythromycin A enol ether (PsEAEN) is formed [12]. Therefore the buffer pH for the extraction solvent was chosen to be 7.0. Erythromycin is slightly soluble in cold water and the solubility was increased by addition of acetonitrile. However, high amounts of acetonitrile in the extraction solvent have to be avoided to limit the extraction of benzoylperoxide. The extraction solvent finally chosen contained 25% of acetonitrile. To neutralise the oxidation power of the extracted benzoylperoxide, ascorbic acid was added to the extraction solvent. Without the addition of ascorbic acid, a peak corresponding to benzoylperoxide was observed in the chromatogram of the extract. Benzoylperoxide absorbs very strongly at a wavelength of 215 nm, which is used for the detection of erythromycin. When the amount of ascorbic acid in the extraction solvent was brought to 1% (m/v), no benzoylperoxide peak was detected. The above resulted in an extraction solvent containing a mixture of 0.2 *M* $K_2HPO_4$, pH 7.0 and 1% (m/v) ascorbic acid brought to pH 7.0 with KOH–acetonitrile (75:25, v/v). The obtained extract remained stable for at least 5 h at a temperature of 5 °C. Therefore an autosampler cooled at 5 °C was used. The LC method for the analysis of erythromycin has been described already [13]. Xterra $RP_{18}$, kept at 65 °C, is used as stationary phase. Isocratic separation was achieved with a mobile phase containing acetonitrile–0.2 *M* dipotassium phosphate, pH 7.0–water (35:5:60, v/v/v), at a flow rate of 1.0 ml/min. The main peak corresponding to EA is eluted at 23 min and is well separated from the other erythromycin and gel components. A typical chromatogram of an extract is shown in Fig. 4. Benzoylperoxide is eluted at ~46 min but is not detected when the above extraction procedure is applied.

A second extraction procedure was developed for the analysis of benzoylperoxide. The solution was 20 times more diluted than for erythromycin and de-

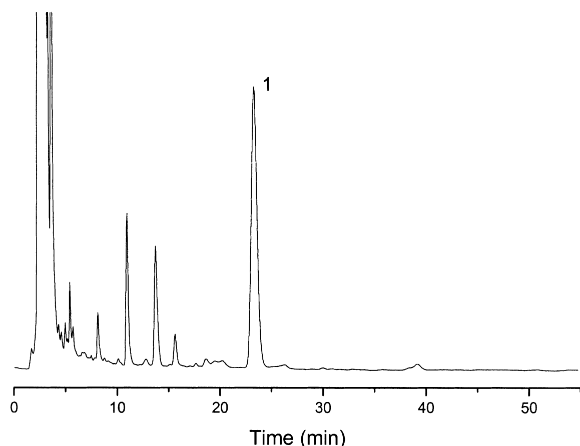


Fig. 4. Typical chromatogram of an erythromycin extract. Peak number 1 is the peak corresponding to EA. Conditions as described in Materials and methods.

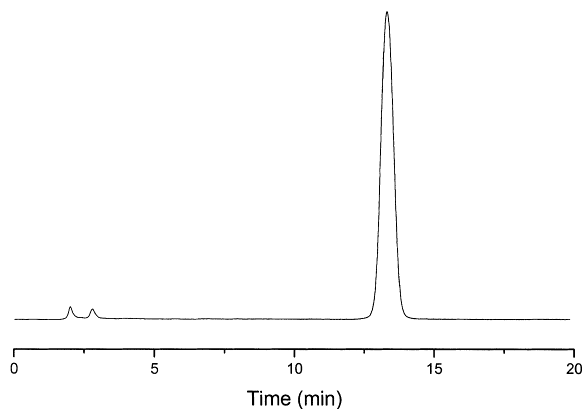


Fig. 5. Typical chromatogram of a benzoylperoxide extract. Conditions as described in Materials and methods.

tection was performed at 254 nm, at which erythromycin does not absorb. The extraction solvent was acetonitrile–water (80:20, v/v). The gel (0.500 g) was stirred in 100 ml of extraction solvent for 30 min, filtered, and the filtrate was diluted ten times with extraction solvent. Using this extraction procedure, the extract remained stable for at least 5 h at room temperature. No decomposition of benzoylperoxide was observed at this temperature. This was proved by addition of increasing amounts of erythromycin to a solution of benzoylperoxide in the extraction solvent. No decrease in benzoylperoxide content was observed. A simple LC method was developed for the analysis of benzoylperoxide. Hypersil BDS $C_{18}$ was used as stationary phase. The mobile phase consisted of acetonitrile–water (58:42, v/v). A typical chromatogram of a gel extract is shown in Fig. 5. The main peak corresponding to benzoylperoxide is eluted at 14 min.

## 3.2. Method validation

### 3.2.1. Repeatability and linearity

The repeatability of the determination of erythromycin and of benzoylperoxide was tested from six extractions and every extract was analysed once. The RSD on the content of erythromycin was 1.7%. The RSD on the content of benzoylperoxide was 2.2%. The linearity of both LC methods was determined in the range of 50–125%, taking 0.3 mg erythromycin/ml extraction solvent and 0.025 mg benzoylperoxide/ml extraction solvent as 100%, respectively. Results of both substances are listed in Table 2.

### 3.2.2. Recovery

The recovery of both erythromycin and of benzoylperoxide was determined. The set-up is shown in Fig. 3. For erythromycin, concentration levels of 75, 100 and 125% and for benzoylperoxide of 70, 100 and 130% were examined. At each concentration level two different matrices were studied. For each matrix, the recovery was estimated at two different days and on each day two replicates were made. This resulted in 12 extractions and 24 different analyses for both erythromycin and benzoylperoxide. The overall recovery $\bar{\bar{R}}_m$ was calculated using Eq. (1). For the determination of erythromycin, $\bar{\bar{R}}_m$ was found to be 0.96 and for the determination of benzoylperoxide 1.01. It was then checked whether or not these overall recoveries were statistically different from 1.

Therefore, a variance analysis was carried out. The ANOVA table (Table 1) shows the mean squares (MS) at each level. From these MS the variances between concentrations, $u(C)^2$, between matrices, $u(M)^2$, between days, $u(D)^2$, and the repeatability variance, $u(r)^2$, were calculated. The variances were

Table 2
Validation results for the analysis of erythromycin and benzoylperoxide

| | Results of the validation | |
|---|---|---|
| | Erythromycin | Benzoylperoxide |
| Repeatability ($n=6$) | RSD 1.7% | RSD 2.2% |
| Linearity<br>$x=50$, 75, 100, 125% ($n=3$)<br>$y=$peak area | $y=38\,521x+92\,851$<br>$R=0.9997$ | $y=1\,067\,500x-2\,127\,500$<br>$R=0.9999$ |
| Recovery | $\bar{\bar{R}}_m=0.96$ | $\bar{\bar{R}}_m=1.01$ |

used in Eq. (6) in order to calculate the intermediate precision, $u(I)^2$. The estimated variances and uncertainties for both the determination of erythromycin and of benzoylperoxide are shown in Tables 3 and 4, respectively. The variances between concentrations and between matrices were found to be negative for both determinations and were set equal to 0 [14]. This means that the recovery is not affected by concentration and matrix variability. The $u(D)^2$ and $u(r)^2$ variances per concentration level were also calculated. The intermediate precision $u(I)^2$ found at each concentration level was then used to calculate the uncertainty of the mean recovery, $u(\bar{\bar{R}}_i)$, at each concentration level, using Eq. (7). The

Table 3
Variances and uncertainties from the recovery experiment for the determination of erythromycin

| Type of variance | Total | 75% | 100% | 125% |
|---|---|---|---|---|
| $u(C)^2$ | $-1.979\times10^{-5}\rightarrow0$ | | | |
| $u(M)^2$ | $-1.855\times10^{-4}\rightarrow0$ | | | |
| $u(D)^2$ | $4.271\times10^{-4}$ | $2.216\times10^{-4}$ | $4.667\times10^{-4}$ | $5.930\times10^{-4}$ |
| $u(i)^2$ | $1.162\times10^{-4}$ | $1.323\times10^{-4}$ | $8.712\times10^{-5}$ | $1.293\times10^{-4}$ |
| $u(I)^2$ | $5.433\times10^{-4}$ | $3.539\times10^{-4}$ | $5.538\times10^{-4}$ | $7.223\times10^{-4}$ |
| $u(\bar{\bar{R}}_i)^{2}$ [a] | | $4.423\times10^{-5}$ | $6.923\times10^{-5}$ | $9.028\times10^{-5}$ |
| $u(\bar{\bar{R}}_i)^{2}$ [b] | | $1.712\times10^{-4}$ | $1.171\times10^{-8}$ | $4.874\times10^{-8}$ |

[a] From intermediate precision [Eq. (7)].
[b] As standard deviation on $\bar{\bar{R}}_i$ [Eq. (4)].

Table 4
Variances and uncertainties from the recovery experiment for the determination of benzoylperoxide

| Type of variance | Total | 75% | 100% | 125% |
|---|---|---|---|---|
| $u(C)^2$ | $-8.612\times10^{-5}\rightarrow0$ | | | |
| $u(M)^2$ | $-1.149\times10^{-4}\rightarrow0$ | | | |
| $u(D)^2$ | $5.361\times10^{-4}$ | $3.972\times10^{-4}$ | $8.885\times10^{-4}$ | $3.225\times10^{-4}$ |
| $u(i)^2$ | $1.796\times10^{-4}$ | $2.012\times10^{-4}$ | $1.286\times10^{-4}$ | $2.088\times10^{-4}$ |
| $u(I)^2$ | $7.156\times10^{-4}$ | $5.985\times10^{-4}$ | $1.017\times10^{-3}$ | $5.313\times10^{-4}$ |
| $u(\bar{\bar{R}}_i)^{2}$ [a] | | $7.481\times10^{-5}$ | $1.271\times10^{-4}$ | $6.641\times10^{-5}$ |
| $u(\bar{\bar{R}}_i)^{2}$ [b] | | $2.043\times10^{-5}$ | $7.411\times10^{-6}$ | $5.663\times10^{-4}$ |

[a] From intermediate precision (Eq. (7)).
[b] As standard deviation on $\bar{\bar{R}}_i$ (Eq. (4)).

uncertainty of this mean recovery can also be calculated as the standard deviation on $\bar{\bar{R}}_i$ using Eq. (4). Results of both approaches for the determination of erythromycin and benzoylperoxide are shown in Tables 3 and 4, respectively. These $u(\bar{\bar{R}}_i)$ were then used in Eq. (3) to estimate the uncertainty of the overall recovery, $u(\bar{\bar{R}}_m)$ (Table 5).

To check whether the recovery is statistically different from 1, $u(\bar{\bar{R}}_m)$ is used in Eq. (2) and the corresponding $t$-value is calculated:

$$t = \frac{\left|\bar{\bar{R}}_m - 1\right|}{u(\bar{\bar{R}}_m)} \tag{8}$$

It should be noted that this test only makes sense if the mean recovery at each concentration level is similar. This can be tested by comparing the ratio of the MS (concentration) to the MS (matrix) by means of an $F$-test. The results in Table 1 show that this $F$-test will not be significant for either the analysis of erythromycin or of benzoylperoxide, as MS (concentration) is smaller than MS (matrix). It is not easy to determine the number of degrees of freedom (df) when $u(\bar{\bar{R}}_m)$ is calculated using the intermediate precision. However from one set-up it can be seen that df will be above 3 and therefore $t_{\mathrm{critical}}$ ($\alpha = 0.05$) will be below 3.18 (3 df) and above 1.96 ($\infty$df). Although the Welch-Satterthwaite approach can be used in order to estimate the exact number of degrees of freedom [15], this approach was not used here since the $t$-values derived were found clearly above or below the critical range given by 3 and $\infty$df. Knowing the exact number of degrees of freedom would lead to a critical value within this range and would lead to exactly the same conclusions as drawn above. For the determination of erythromycin both approaches give a $t$-value higher than $t_{\mathrm{critical}}$. This means that the recovery is statistically different from 1 and the procedure has a significant bias. Therefore a correction factor, equal to the recovery factor, should be applied to future results [9]. However, regulatory bodies may have problems accepting ‘‘corrected’’ results. To overcome this problem the content limits may be adapted according to the correction factor. This allows the recovery factor to be taken into account without the need to adapt all future results. For the determination of benzoylperoxide, the recovery is found not to be statistically different from 1.

The uncertainty arising from the precision of the method, calculated as $u(I)/R_m$, can be calculated as well and is found to be $2.43\times10^{-2}$ (or 2.43%) for erythromycin and $2.65\times10^{-2}$ (or 2.65%) for benzoylperoxide.

### 3.3. Sample analysis

To check the stability of both erythromycin and benzoylperoxide in Benzamycin gel, a stability study was carried out over a period of 2 months. One batch of each type of carbomer (940 and 980) was used. Of each batch two gels (940.1, 940.2 and 980.1, 980.2) were prepared. For the analysis of erythromycin and of benzoylperoxide, two extractions were carried out at each time point and every extract was injected twice. Five time points were chosen: $T=0$, the first analysis of the gel was performed immediately after mixing erythromycin in the gel, $T=1$, after 1 week, $T=2$, after 2 weeks, $T=3$, after 4 weeks and $T=4$, after 8 weeks. Samples were stored in the refrigerator at 3 °C. At each time point the contents of erythromycin and of benzoylperoxide were tested. All results are summarized in Table 6. The results shown are the mean and the RSD of four

Table 5
Uncertainty on the overall recovery $u(\bar{\bar{R}}_m)$ and $t$-test

| Method | $u(\bar{\bar{R}}_m)$ | $t$ | df | $t_{\mathrm{critical}}$ |
|---|---|---|---|---|
| Erythromycin | $8.241\times10^{-3}$ [a] | 4.97 | >3 | $1.96<t_{\mathrm{crit}}<3.18$ |
| | $7.555\times10^{-3}$ [b] | 5.43 | 3 | 3.18 |
| Benzoylperoxide | $9.458\times10^{-3}$ [a] | 1.14 | >3 | $1.96<t_{\mathrm{crit}}<3.18$ |
| | $1.407\times10^{-2}$ [b] | 0.77 | 3 | 3.18 |

[a] From intermediate precision results.
[b] Based on standard deviation on $\bar{\bar{R}}_i$.

Table 6
Sample analysis: results of the stability study

| | Erythromycin content, % ($n=4$) | Benzoylperoxide content, % ($n=4$) |
|---|---|---|
| *Gel 940, sample 1* | | |
| $T=0$ | 111.5 (RSD 3.2%) | 105.1 (RSD 1.5%) |
| $T=1$ | 109.8 (RSD 1.8%) | 106.9 (RSD 2.2%) |
| $T=2$ | 117.1 (RSD 1.1%) | 105.2 (RSD 7.0%) |
| $T=3$ | 111.5 (RSD 5.0%) | 93.3 (RSD 7.6%) |
| $T=4$ | 113.0 (RSD 0.6%) | 93.2 (RSD 1.6%) |
| *Gel 940, sample 2* | | |
| $T=0$ | 106.9 (RSD 1.7%) | 104.9 (RSD 1.8%) |
| $T=1$ | 101.5 (RSD 3.8%) | 106.4 (RSD 3.2%) |
| $T=2$ | 111.0 (RSD 1.4%) | 111.8 (RSD 2.4%) |
| $T=3$ | 106.0 (RSD 1.5%) | 101.9 (RSD 2.7%) |
| $T=4$ | 109.9 (RSD 1.7%) | 113.6 (RSD 5.6%) |
| *Gel 980, sample 1* | | |
| $T=0$ | 109.7 (RSD 0.7%) | 105.4 (RSD 1.8%) |
| $T=1$ | 110.7 (RSD 2.7%) | 104.0 (RSD 0.3%) |
| $T=2$ | 109.6 (RSD 0.5%) | 101.8 (RSD 9.6%) |
| $T=3$ | 100.3 (RSD 4.3%) | 98.2 (RSD 3.2%) |
| $T=4$ | 102.0 (RSD 7.3%) | 104.6 (RSD 0.8%) |
| *Gel 980, sample 2* | | |
| $T=0$ | 112.5 (RSD 3.3%) | 111.5 (RSD 6.6%) |
| $T=1$ | 115.0 (RSD 0.2%) | 110.4 (RSD 1.8%) |
| $T=2$ | 112.0 (RSD 2.4%) | 103.5 (RSD 2.1%) |
| $T=3$ | 108.5 (RSD 5.7%) | 101.4 (RSD 0.9%) |
| $T=4$ | 115.6 (RSD 1.4%) | 99.1 (RSD 3.9%) |

$T=1$, 1 week; $T=2$, 2 weeks; $T=3$, 4 weeks; $T=4$, 8 weeks.

injections. The results for the erythromycin content are not corrected by the recovery factor. The amount of erythromycin added in the gel corresponded to 110% (3.3%, m/m). For erythromycin the starting material used for the preparation was taken as the reference substance (content: 100%). USP content limits for erythromycin and benzoylperoxide in topical preparations are 90–125% [16]. These wide limits already take into account the extraction procedure. The samples comply with these limits over the examined time interval, even if the recovery factor, which lowers the content limits, is not taken into account. The erythromycin content is between 100 and 117%. Variability in precision and content can be explained by heterogeneity of the gel. No formation of the oxidation product, erythromycin A N-oxide, could be detected. For benzoylperoxide a commercial sample was used as the reference substance. Its content (86.11%) was determined by iodometric titration. The benzoylperoxide content of the gels is between 93 and 112%. It can be concluded that Benzamycin is stable over a period of at least 2 months when stored in a refrigerator. No difference was observed between type 940 and type 980.

## 4. Conclusion

Efficient LC methods for the analysis of erythromycin and benzoylperoxide in gel were developed. The stability of the sample solution was ensured by using an appropriate extraction solvent. Both methods showed good selectivity, repeatability and linearity. The recovery of both methods was examined and a method to evaluate the occurrence of bias in recovery experiments was demonstrated.

Using this method the stability of four samples was shown over a time interval of 2 months.

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