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## Stability of alemtuzumab in infusion-bags

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To determine the physical and chemical stability of alemtuzumab a high-performance-liquid-chromatography-method was developed. The antibody was stored over 14 days at 6 °C, at room temperature and on a vibrating plate and tested by size-exclusion chromatography (SEC) using a phosphate buffer (0.1 M, pH 7) with 0.3 M sodium chloride. The method was also used to quantify alemtuzumab and was validated by parameters such as linearity, range, limit of quantification (LOQ), limit of detection (LOD) as well as precision and robustness. The physical and chemical stability of alemtuzumab could be demonstrated for a time-period of 14 days.

The monoclonal antibody alemtuzumab is used for the treatment of chronic lymphocytic leukaemia as a second line therapy for patients who have been treated with alkylating agents and who have failed fludarabine therapy (Barth 2002). The commonly used concentration of alemtuzumab in infusion of 0.9% aqueous sodium chloride is 0.3 mg/ml. To obtain this concentration the commercially available preparation is to be diluted. The stability for dilutions of Alemtuzumab in 0.9% aqueous sodium chloride is limited by the manufacturer up to 8 h when stored at room temperature or in the refrigerator (Fachinformation MabCampath). Since storage and application duration can exceed the limited time interval stability needs to be further investigated. Therefore, this study evaluated the chemical and physical stability of alemtuzumab in infusion-bags.

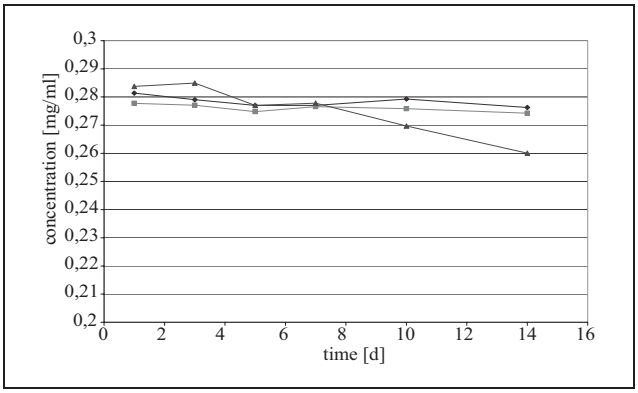


Fig.: Concentrations of alemtuzumab in 0.9% aqueous sodium chloride solution over a time period of 14 days; primary packaging material 100 ml freeflex®-bags; KS —●— refrigerator, RT —■— room temperature, SP —▲— vibration plate at room temperature

The infusion-bags were incubated over a time period of 14 d at 6 °C, room temperature and at a vibrating plate at room temperature to simulate the effects of transport. Samples were withdrawn at selected intervals and analyzed by SEC-HPLC. The concentration measured on day one was 0.28 mg/ml and the pH was 6.88.
Samples stored in bags showed only a small fall of concentration during the maintained period except for the samples on the vibrating plate (Fig.). However the concentration of alemtuzumab remained over the 90 percent level for the samples stored at 6 °C, room temperature and vibrating plate, demanded by the APV guidelines (APV 1985).

## Experimental

The test solution was prepared using the commercially available preparation, MabCampath® ampoule, alemtuzumab 30 mg/ml (Batch number: 63027E, Schering AG). For packaging 100 ml freeflex®-bags (Fresenius Kabi AG, Bad Homburg) filled with 0.9% aqueous sodium chloride were used. The test solution was prepared by adding 1 ml drug solution to 100 ml freeflex®-bags and mixed thoroughly. The concentration in each bag was 0.28 mg/ml. After preparation samples of contents of all bags were withdrawn and analyzed. The mean represents the concentration of the preparation day, shown as day one.
The bags were incubated at 6 °C, room temperature and at a vibrating plate at room temperature over a time period of 14 days. Samples were withdrawn at days 1, 3, 5, 7, 10 and 14 and analyzed in duplicate. Four samples at every storage location were used: One 100 ml infusion-bag, two 50 ml bags with 0.29 mg/ml alemtuzumab and a blind-sample filled with 0.9% aqueous sodium chloride to test the change of pH in the matrix. One 50 ml infusion-bag was only analysed at day 1 and 14 to eliminate errors caused by contaminations and repeated withdrawal. Another 50 ml infusion-bag was used as comparison for the quantification of alemtuzumab. Antibody solution (50 µl) was used for HPLC. The concentration of the samples was obtained by SEC-HPLC.
HPLC was performed on a Dionex instrument equipped with an ASI-100 autosampler and a 170S UV-VIS detector set at 280 nm. For data acquisition Chromeleon™, version 6.20 (Dionex) was used. The antibody was separated on a TSK-Gel® G 3000 $SW_{XL}$ 30 cm × 7.8 mm ID column (Tosoh Bioscience, Stuttgart). The mobile phase was a mixture of 0.3 M NaCl in a phosphate buffer (0.1 M, pH 7.0). The flow rate was set at 1.0 ml/min. UV detection was performed at 280 nm. Quantification of the analyte was based on the peak area ratio method. All analyses were performed at 25 °C warranted by a column oven STH 585 (Dionex).
The method was validated with respect to linearity, range, limit of quantification (LOQ), limit of detection (LOD) as well as precision and robustness. The terms are used according to the definition of the ICH guideline Q2(R1) (ICH 2005). Alemtuzumab was calibrated in the concentration range 0.06 mg/ml to 0.375 mg/ml. The calibration curve was constructed from six different concentrations. Each concentration was prepared in triplicate. Linear relationship was obtained. The lowest concentration which was detectable was assayed at $1.08 \times 10^{-3}$ mg/ml. This is shown as the LOD for this assay. The LOQ defined as an x-value at which the relative standard deviation just reaches an acceptable threshold of 10% was $4.28 \times 10^{-3}$ mg/ml. By analyzing a solution of approximately 0.3 mg/ml on six independent series on the same day and on six consecutive days lead to the repeatability and intermediate precision of this method. The measurement of daily new prepared 0.3 mg/ml solutions on six days delivered the intra-laboratory-precision. The obtained data are listed in the Ta-

**Table: Intra-day precision, inter-day precision and intra-laboratory precision of alemtuzumab**

| | c (mg/ml) | s (mg/ml) | RSD (%) |
|---|---|---|---|
| Intra-day precision | 0.298 | 0.00032 | 0.105 |
| Inter-day precision | 0.304 | 0.00056 | 0.186 |
| Intra-laboratory precision | 0.300 | 0.00204 | 0.680 |

ble. The robustness could be demonstrated for changes of pH between 6.8 and 7.2, molality of NaCl between 0.25 and 0.35 M and temperature between 20 and 30 °C.
The results show that this method is selective and sensitive and therefore suitable for analyzing alemtuzumab in aqueous solution. Also a physical and chemical stability of Alemtuzumab was shown for a time-period of at least 14 days.

**References**

Barth J (2002) Alemtuzumab – Zytostatika-Monographie der Arbeitsgruppe Onkologie im ADKA-Ausschuss Klinische Pharmazie (AO), Krankenhauspharmazie 23: 344–345.
FACHINFORMATION: MabCampath® (2006) Bayer Schering Pharma AG
ICH (2005) Harmonised Tripartite Guideline: Validation of Analytical Procedures: Text and Methodology Q2(R1), Current Step 4 version.
Apv (1985): Haltbarkeit und Haltbarkeitsprüfung von Arzneimitteln. Apv-Richtlinie mit Kommentar, Pharm Ind 47 (6).

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## A novel sesquiterpene from the roots of *Vladimiria souliei*

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A novel sesquiterpene lactone, 1β, 14-peroxy-4α-hydroxy-5αH, 7αH,6βH-eudesm-11(13)-en-6, 12 olide (**1**), was isolated from the roots of *Vladimiria souliei*. The structure was elucidated by spectroscopic methods.

*Vladimiria souliei* (Franch.) Ling is a medicinal plant mainly distributed in Sichuan Province, China. Its roots, a traditional Chinese medicine, have been used for relieving pain and stomach diseases since ancient times (Editorial commission of Traditional Chinese Medicine 1999). Previous phytochemical investigations on this species resulted in the isolation of sesquiterpene lactones (Tan et al. 1990a), and ligans (Tan et al. 1990b). In the course of our study, a new sesquiterpene lactone was isolated from the roots of *Vladimiria souliei* (Franch.) Ling. The structure was elucidated by spectroscopic methods. In this paper, we present the isolation and structural elucidation of the new sesquiterpene lactone based on the spectral analysis.
Compound **1** was obtained as colorless oil. Its ESI-MS spectrum showed a quasi molecular ion peak at m/z: 303 $[M + Na]^+$. Its molecular formula was determined as $C_{15}H_{20}O_5$ from its HR-ESI-MS spectrum (m/z 303.1217 $[M + Na]^+$, Calcd for $C_{15}H_{20}O_5Na$, 303.1208).
The $^1$H NMR showed the olefinic protons of the double bond at δ 6.12 (1 H, d, J = 3.1 Hz, H-13a), δ 5.46 (1 H, d, J = 3.1 Hz, H-13b) along with one methyl at δ 1.49 (3 H, s, H-15). In addition, four protons of oxygenated carbons at δ 4.49 (1 H, br. s, H-1), 3.63 (1 H, t, J = 10.8, 10.8 Hz, H-6), 4.19 (1 H, d, J = 6.2 Hz, H-14a), 5.00 (1 H, J = 6.2 Hz, H-14b) and other protons in the upfield region were also observed in the $^1$H NMR spectrum of compound **1**. The $^{13}$C NMR spectrum revealed 15 carbon

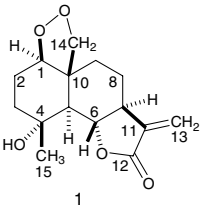


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Fig. 1: Chemical structure of compound **1**