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A study of interlaboratory influence on column evaluation

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Abstract

A liquid chromatography method for the characterization of base deactivated columns was investigated in a collaborative study involving six laboratories. This work was carried out on two chromatographic supports (Xterra RP 18 and Symmetry Shield). Different cooling systems, namely water bath and air oven, were tested and it was shown that column thermoregulation did not significantly influence chromatographic data. In order to control the mobile phase composition, the latter was prepared by weight rather than volume. Thanks to the injection of a set of selected neutral compounds, extra-column effects were evaluated in each of the participating laboratories. The results showed that chromatographic supports tested in different laboratories and following the same test protocol could be effectively compared.

Keywords: Interlaboratory study; High-performance liquid chromatography; Basic compounds; Neutral compounds

1. Introduction

Interlaboratory studies are considered as an important aspect in method validation during an analytical transfer. Besides reproducibility, it is essential to determine whether factors such as "laboratory equipment" or "preparation of mobile phases" introduce significant result dispersion [1,2]. For that purpose, it is necessary to perform a collaborative study involving at least six laboratories using the same procedure when analysing the same products [1]. Effectively, data stemming from a single laboratory are in no way sufficient to estimate method reproducibility [3].

In this work, a collaborative study was undertaken by six laboratories following the same experimental protocol related to a chromatographic test for the evaluation of base deactivated chromatographic columns [4,5]. All experiments were carried out with the same chromatographic columns to avoid possible variations due to the stationary phase. Each laboratory used its regular HPLC equipment. Buffer salts and mobile phase solvents were provided from local sources. The generating laboratory (laboratory 1) was in charge of providing test solutions in sealed ampoules to each participant.

A set of 10 (five neutral and five basic) test compounds was selected. Basic compounds were chosen to assess interlaboratory variability of retention, and asymmetry factors were used for the evaluation of base deactivated supports. Neutral compounds were included for the estimation of extra-column effects. In addition, the possible correlation between the nature of the analyte and interlaboratory variability was investigated for all measured chromatographic parameters (k, As and N).

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As already reported in the literature, column thermoregulation system is one of the main variables affecting interlaboratory variability [6]. Therefore, a comparison between air oven and water bath thermoregulation system was also performed by a limited number of laboratories.

Laboratory 1 was in charge of testing chromatographic supports at the beginning and at the end of the study to evaluate possible column performance deterioration.

In addition, for one of the two tested chromatographic supports (Xterra RP 18), inter-batch variability ($n = 4$) data, measured in the same laboratory, were determined and compared to interlaboratory data ($n = 6$).

2. Experimental

2.1. Chemicals and materials

The test solutes for this interlaboratory study were of analytical reagent grade. Chloroprocaine hydrochloride (CL) was provided by Orgamol (Evionnaz, Switzerland). Diphenhydramine hydrochloride (DP) and codeine (CO) were supplied by Siegfried (Zofingen, Switzerland). Fentanyl citrate (FN) was from Mcfarlan Smith Limited (Edinburgh, Scotland) and quinine hydrochloride (QN) from Hänseler AG (Herisau, Switzerland). All neutral compounds were obtained from Merck (Darmstadt, Germany), Fluka (Buchs, Switzerland), Janssen (Beerse, Belgium), Aldrich (Steinheim, Germany) at the highest available purity.

Acetonitrile was from SDS (Peypin, France). Water was obtained with the Milli-Q Water Purification System from Millipore (Milford, MA, USA). Aqueous buffer was prepared with di-potassium hydrogen phosphate anhydrous and potassium di-hydrogen phosphate (Fluka-Buchs, Switzerland) by measuring the pH with a Metrohm pH meter (Herisau, Switzerland).

Principal component analysis was performed with the Simca P software package (Umetrics, Sweden).

2.2. Test solutions and columns

Each laboratory was provided with sealed ampoules containing the test solutions, which were simply diluted to 10 ppm in the mobile phase immediately before injection in the HPLC system.

The participants received two columns: a Symmetry Shield (150 mm × 4.6 mm i.d., 5 μm) and an Xterra RP18 (150 mm × 4.6 mm i.d., 5 μm), both manufactured by Waters® (Milford, USA). Both columns were sent from laboratory to laboratory to make sure that all possible interlaboratory differences were not due to column (or batch) variability.

2.3. Conditions and procedure

In order to assess the most realistic estimation of interlaboratory variability, each participating laboratory was asked to use its routine HPLC equipment. A list of general criteria was specified concerning operating conditions. As an example, data acquisition rate and detector response time were, respectively, fixed at 20 Hz and 0.1 s. Both equilibrating and cleaning procedures were specified in the analytical protocol. The mobile phase was prepared by weight rather than volume and it was composed of acetonitrile – pH 7.0, 0.0375 M phosphate buffer (31.1:60.0 w/w), corresponding to (40:60; v/v) of reference [4]. The injection sequence was randomised for each laboratory. The detection wavelength was set at 215 nm, flow rate at 1.0 ml/min and analyses were carried out at 30 °C.

Three chromatographic parameters (k, As and N) were measured according to the following equations:

Retention factor:

$$k = \left(\frac{t_r - t_0}{t_0}\right) \quad (1)$$

where t_r was the compound retention time and t_0 the column void volume retention time (measured with $NaNO_3$);

Asymmetry:

$$As = \frac{1}{2} \times \left(1 + \frac{B}{A}\right) \quad (2)$$

where A and B were evaluated at 5% of the peak height;

Efficiency:

$$N = 5.54 \times \left(\frac{t_r}{w_{1/2}}\right)^2 \quad (3)$$

where $w_{1/2}$ was the peak width at 50% of the peak height.

3. Results and discussion

According to the previously developed chromatographic test [5], column performance was assessed by measuring retention (k) and asymmetry factors (As) of a reduced number of basic test compounds. Moreover, neutral compounds (*N*,*N*-diethylacetamide, phenol, nitrobenzene, anisole and naphthalene) were included to determine extra-column effects in this study. Interlaboratory variability ($n = 6$) of k and As was calculated in terms of relative standard deviation (R.S.D., in %) for each test compound on both stationary phases.

3.1. Estimation of extra-column effects

It is well known that extra-column volumes, such as tubing, injector, detector cell, etc. can decrease chromatographic performance. Therefore, the observed efficiency, N_{obs}, determined from Eq. (3) may be lower than the actual column efficiency, N_{col}, particularly for the less retained solutes. For this reason, the set of neutral compounds selected for this collaborative study was injected by each participating laboratory to estimate extracolumn effects. For a given compound, the