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Argentometric Titration for the Determination of Liquid Chromatographic Injection Reproducibility

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The reproducibility of autosamplers for high-performance liquid chromatography can be checked by the injection of a sodium chloride solution without an installed column. The dispensed chloride plug is analyzed quantitatively by argentometric titration. If the titration can be performed with high enough precision, the autosampler performance is investigated without additional effects. Experiments with a 20-μL loop were performed. We found the following repeatabilities (relative standard deviations): titration, 0.07%; 10-μL "pull loop" injection (partial filling of the loop), 0.6%; and 20-μL "full loop" injection, 0.1%. In contrast to these data, the relative standard deviations obtained from chromatographic peaks include the steps of injection, separation, detection, and integration without any knowledge of the individual contributions. Such values are higher; we found for chromatographic peak areas of parabens 2% under pull loop conditions and 0.17% by full loop. The titration approach allows the determination of the contribution of the injection to the uncertainty budget of a liquid chromatographic analysis.

Liquid chromatographic procedures often suffer from rather poor precision in the 1−2% range of relative standard deviation (RSD). For instrument manufacturers and for research in the field of measurement uncertainty, it is highly desirable to develop techniques to find out the weak steps of the procedure in order to find ways to improve the precision. In the course of the instrument test for high-performance liquid chromatography (HPLC) systems, the autosampler injection reproducibility is generally checked by n (e.g., 6 or 10) replicate injections and chromatographic separation of a test analyte or a test mixture.[1] Usually the peak areas are evaluated. This is a realistic and easy-to-perform test, although it determines the combined standard uncertainties of the injection, u_{inj}; of the separation process on the column, u_{sep}; of the detection, u_{det}; and of the integration, u_{int}. The total relative standard uncertainty of the chromatographic process u_{chrom} is the square root of the sum of relative variances

$$u_{chrom,rel} = \sqrt{u_{inj,rel}^2 + u_{sep,rel}^2 + u_{det,rel}^2 + u_{int,rel}^2} \qquad (1)$$

Therefore, no information about the performance of the individual parts of the chromatograph can be obtained by the determination of u_{chrom}. For the test analyte for the separation on a reversed-phase system, one can choose a convenient compound such as caffeine or a more troublesome one such as benzophenone; quite often the RSD as determined by using benzophenone will be slightly higher than with caffeine.[2] Thus, caffeine can yield a value that is too optimistic for HPLC reproducibility, as compared to the "real-life" separations, due to its excellent properties as a solute and a chromatographic analyte. Some laboratories use thiourea as the test analyte because it is not retained under the usual conditions of reversed-phase HPLC. However, nonretained compounds can give rise to very low or untypically high-peak-area RSDs.[3]

In the course of an instrument test the true contribution of the autosampler alone is never evaluated, and it is assumed that the chromatographic data really represent the injection precision.[4] A large number of data from industrial quality control are presented by Renger.[5] The autosampler can, indeed, be a weak part in the uncertainty budget because it is an assembly of many mechanical parts and a number of error sources can affect its operation.[6] It can be used in two different modes (and variations thereof), namely, partial or complete filling of the loop, and it is well-known that complete filling (i.e., overfilling) results in markedly higher precision.[7]

For the investigation of the autosampler performance without the additional effects of separation, detection, and integration, it is necessary to use a nonchromatographic method. We describe here our experience with the argentometric titration of a sodium chloride test solution. This is an ideal analyte because an argentometric titration curve is steep, the end-point can be determined unambiguously with a silver electrode, and environ-

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mental influences such as carbon dioxide or humidity do not affect the result of the analysis.

EXPERIMENTAL SECTION

Analytes. The test compound was sodium chloride "pro analysi" (Merck, Darmstadt, Germany). Its purity was indicated as ≥99.5%, but this number is not of importance because only RSDs have been determined in our study. For the same reason, the concentration of the test solution need not be known exactly. Solutions of approximately 10% (m/v) of sodium chloride in ultrapure water were prepared and used for the tests (10 μL corresponds to approximately 1 mg NaCl).

For the additional chromatographic tests, a mixture of methyl, ethyl, propyl, and butyl paraben with a concentration of approximately 1 mg/L each in mobile phase was prepared (Fluka, Buchs, Switzerland).

HPLC System for the Investigation of Sodium Chloride Samples. For this study, a Spectra-Physics instrument was used (Spectra-Physics Analytical, Fremont, CA). The pump was a SpectraSystem P4000 with low-pressure gradient mixer; the autosampler was a SpectraSystem AS3000 with a 20-μL loop. The mobile phase was ultrapure water at a flow rate of 1 mL/min. The autosampler was used in two different modes: under pull loop conditions, the loop was filled partially with 10 μL of sample solution, whereas it was filled totally (i.e., overfilled) in the full loop mode. Instead of a column, a backpressure regulator was installed which provided a pressure of approximately 100 bar (Alltech, Deerfield, IL). At its outflow, a short capillary led to a glass beaker where the eluted NaCl plug was collected manually during approximately one-half of a minute. The beakers were transferred directly into the titration system. A series of experiments consisted of 10 replicate injections of constant volume and 2 blank analyses (i.e., mobile phase which was collected during the same time interval) as controls for the proper function of the titration system.

HPLC System for Paraben Samples. The same HPLC instrument was used, but the backpressure regulator was replaced by a 12.5 cm × 3 mm i.d. column with Nucleosil 100 C18, 5-μm reversed-phase stationary phase (Macherey-Nagel, Oensingen, Switzerland). The mobile phase was water/acetonitrile 65:35 (v/v) with a flow rate of 1 mL/min. The mixture was generated within the low-pressure gradient valve of the pump. Detection was performed using a Spectra Focus UV detector at 254 nm (Spectra-Physics Analytical). For data acquisition and integration, TSP PC1000 System software was used (Thermo Separation Products, Fremont, CA). Again, the injection techniques pull loop and full loop using a 20-μL loop were tested, and 10 replicate separations from the same vial were performed.

Titration System. For the titrimetric analyses, the sodium chloride samples were diluted with approximately 25 mL of ultrapure water and 30 mL of acetone and acidified with 5 mL of 0.25 M nitric acid. The addition of acetone decreases the solubility product of silver chloride, which results in a lower detection limit for chloride. The titration reagent was 0.005 M silver nitrate solution (diluted from 0.1 M, Titrisol Merck, with ultrapure water). As described above, the concentration of the sodium chloride solution does not need to be exactly known for the determination of RSD's. The analyses were performed in a thermostated laboratory at 296 K (23 °C). The titrator was a 736 GP Titrino with a 10 mL dosing unit, the electrode was a Ag Titrode (both from Metrohm Ltd., Herisau, Switzerland). The performance of the titration was checked with a sodium chloride solution having approximately 720 μg NaCl/mL. Of this solution, aliquots of 3 mL were transferred using a 700 Dosino equipped with a 5-mL dosing unit (Metrohm) into titration glass beakers and diluted with water, acetone, and nitric acid as described above. Ten replicate samplings and titrations were performed.

RESULTS AND DISCUSSION

The main prerequisite for a titrimetric autosampler test is that the analyses can be performed with markedly higher precision, as compared to the injector repeatability. We found a RSD of 0.07% when 3-mL samples of sodium chloride solution (with approximately 2.2 mg NaCl) were dosed and titrated by the procedure described in the Experimental Section (needing 7.4073 ± 0.0050 mL of silver nitrate solution). To obtain this high precision, special care is needed (e.g., thermostating of the laboratory and the exclusion of air bubbles from all tubings), but such a value is not uncommon with argentometric titration,[8] which is a rugged method. The RSD of the titration of reference samples represents the combined RSDs of the volumes expelled by the dispenser (the Dosino) and the titration buret (the Titrino) together with the end point determination by the electrode

$$u_{\mathrm{tit,ref,rel}} = \sqrt{u_{\mathrm{dis,rel}}^2 + u_{\mathrm{bur,rel}}^2 + u_{\mathrm{EP,rel}}^2} = 0.0007 \text{ or } 0.07\% \quad (2)$$

For the analyses of samples generated by the HPLC autosampler, the parameter u_{dis} does not appear in the above equation. This means that the relative uncertainty of the titration system alone is even lower

$$u_{\mathrm{tit,rel}} = \sqrt{u_{\mathrm{bur,rel}}^2 + u_{\mathrm{EP,rel}}^2} < 0.07\% \quad (3)$$

The 10-μL pull loop samples, as dispensed by the autosampler, needed 3.5749 ± 0.0228 mL of silver nitrate solution to reach the end-point. The 20-μL full loop samples needed 7.4951 ± 0.0091 mL. This gives RSDs of 0.64 and 0.12%, respectively. (Note that we did not intend to investigate the accuracy of injection or loop volume under various conditions; neither did we take care that the sodium chloride solutions of the two injection series had exactly the same concentration.) These RSDs, the relative uncertainties of the titration of autosampler solutions, also represent the square root of a sum of variances

$$u_{\mathrm{tit,AS,rel}} = \sqrt{u_{\mathrm{inj,rel}}^2 + u_{\mathrm{tit,rel}}^2} \quad (4)$$

If we estimate that $u_{\mathrm{tit,rel}}$ is 0.05% (although there is no proof for this number), it is possible to calculate $u_{\mathrm{inj,rel}}$

$$u_{\mathrm{inj,rel}} = \sqrt{u_{\mathrm{tit,AS,rel}}^2 - u_{\mathrm{tit,rel}}^2} \quad (5)$$

We obtain as relative injection uncertainties for the pull loop and full loop techniques, respectively,

(8) Strohm, D. Metrohm Ltd., Herisau, Switzerland. Personal communication, April 27, 2000.

$$u_{\text{inj,PL,rel}} = \sqrt{0.64^2 - 0.05^2}\% = 0.64\% \quad (5a)$$

$$u_{\text{inj,FL,rel}} = \sqrt{0.12^2 - 0.05^2}\% = 0.11\% \quad (5b)$$

It becomes obvious that a titration RSD ($u_{\text{tit,ref,rel}}$) of 0.07% is adequate for the determination of injection RSDs down to the 0.1% range because its contribution to the combined uncertainty, $u_{\text{inj,rel}}$, is neglectibly small. RSDs of the titration can be expected to be lower if larger sodium chloride samples are analyzed; for example, samples from a 100-μL loop which are in the 10-mg range, because a silver nitrate solution of higher concentration can be used and the titration curve will be steeper.

In contrast to these investigations of autosampler precision by titration, we obtained worse RSDs from chromatographic experiments. With the pull loop technique, the four parabens gave peak area RSDs between 1.7 (methyl paraben) and 2.2% (butyl paraben) and peak height RSDs between 1.8 and 3.3%. In the full loop mode, we found RSDs between 0.14 and 0.24% for areas and 1.1−1.9% for heights. Such numbers open a wide field of speculation about how the parameters injection, separation, detection, and integration interact together to give these data, and it becomes obvious that sophisticated methods are needed if the contribution of each parameter is to be investigated. Short-term flow deviations affect peak areas if a concentration-sensitive detector such as UV is used, but peak heights are influenced by variations of mobile phase composition as a result of variations in the retention factor. There is not much in-depth knowledge about the flow constancy of pumps and the reasons for poor performance. An exception is the results obtained by Siffrin.[9] In our study, the early eluted peaks had better RSDs than the later ones, but the reason, therefore, cannot be found without additional experiments. The signal-to-noise value of the smallest peak (butyl paraben in the pull loop mode) was approximately 100. For the investigation of the performance of the data system it would be necessary to feed the integrator with artificial signals of defined shape and signal-to-noise value.

The *accuracy* of an injection (i.e., the calibration of the loop volume in the case of complete filling or the accurate positioning of a syringe plunger in the case of partial filling) is usually not of concern in HPLC analyses because calibration by the injection of reference solutions is needed. For most analytical runs, the same volume of reference and sample solutions is injected in close temporal vicinity,; therefore, only a good repeatability is important. *Errors* must be recognized as such and avoided; for example, a too tight or too loose closure of the vials, wrong positioning of vials or injector needle, too rapid sample transfer from vial to loop in the case of viscous solutions, etc.

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CONCLUSIONS

By titration experiments, it is possible to determine the precision of injection without the influence of chromatographic, detection, or integration processes. A prerequisite is the ability to perform titrations at a lower relative standard deviation than the autosampler operation. We recommend the use of sodium chloride as the test analyte and silver nitrate as the titration reagent.

The final goal of such studies is to develop methods for the independent investigation of all parameters which influence the precision of liquid chromatographic analyses. If the uncertainty budget of such analyses would be known in detail, that is, if we had knowledge of the contributions of injection, separation, detection, and integration, it would be possible to identify the main sources of uncertainty. This would allow engineers and scientists to concentrate their efforts on the weak parts or processes within an HPLC instrument. Our study leads to the assumption that injection is not the weak point of a liquid chromatographic analysis if an excellent instrument and the appropriate injection technique is used, although the question remains open whether the injection of organic solvents can be performed with the same precision as aqueous solutions. Then a large RSD of an HPLC analysis has other reasons.

ADDENDUM: ALTERNATIVE TEST PROCEDURES FOR THE DETERMINATION OF INJECTION PRECISION

Another possibility of injection precision testing would be to use analytes with a distinct UV or visible spectrum and to determine their concentration by spectroscopy. This approach requires the precise dilution to the mark of a volumetric flask at constant temperature and an absorption measurement. If a single-step dilution to 100 mL of water is made, a RSD of 0.07% can be expected for the dilution alone[10] (multistep dilution by using more than one volumetric flask and pipets would give a higher RSD). The determination of UV or visible absorption should be possible with high precision if all the measurements are made in a directly consecutive procedure.

Weighing of the autosampler vial before and after injection is an approach used by Hansen et al.[11] An injection precision of 0.6% was found (standard uncertainty 0.6 μL with an injection volume of 100 μL). The standard uncertainty of such a weighing operation can be calculated[12] if the technical parameters of the balance are known. Assuming a repeatability of rep = 0.015 mg and a linearity of lin = 0.03 mg (which are the parameters of a Mettler AT 201 balance),[13] the standard uncertainty is obtained as follows

$$u_{\text{weighing}} = \sqrt{u_{\text{rep}}^2 + 2(u_{\text{lin}})^2} = \sqrt{0.015^2 + 2\left(\frac{0.03}{\sqrt{3}}\right)^2}\ \text{mg} = 0.03\ \text{mg} \quad (6)$$

Linearity must be handled as a rectangular distribution; therefore, the linearity term needs to be divided by $\sqrt{3}$. The result indicates that weighing differences of 0.6 mg can be determined with high enough precision if such a balance is used.

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