

# Sustainability in motion: Investigation of automated gravimetric sample preparation in industrial liquid chromatography

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## ABSTRACT

For analysis with liquid chromatography (LC), samples and calibration standards generally require a dilution by a factor of  $10^3$  to  $10^6$ . To guarantee a high accuracy, sample preparation usually employs high-volume pipettes and volumetric flasks for dilution series. Consequently, sample preparation is a prominent driving factor for consumption of solvents in the LC laboratory. Miniaturisation in sample preparation can thus be a means of reducing the required amount of solvent within the laboratory, saving valuable resources. In the context of dilution series, this can be achieved by the use of low-volume dispensing tools, which usually have a higher relative instrument error, resulting in a less accurate overall method. Another approach is the transition to a gravimetric sample preparation, in which the dilution steps are not measured in volume but weight, only depending on the much lower error of the analytical balance. By implementing weighing robots, one can fully automate the sample preparation workflow. This study deals with the comparison of various dilution methods. Gravimetric, robot-aided dilution allows for the reduction of the solvent down to the amount of sample needed for analyses. Including the initial dissolution of the sample, using gravimetric dilution can reliably and repeatedly reduce the required solvent amount by over 90 %, while still generating the same analytical results. Overall, this application leads to significant economic, ecological, social, and technological benefits for the LC laboratory.

## 1. Introduction

Discussion of liquid chromatography (LC) in the context of green analytical chemistry is impossible without addressing the amount of organic solvent consumed before and during analyses. Generally, there are two ways to address this, reduction and replacement, with the underlying challenge of not negatively affecting the analytical performance of the method [1,2]. In cases in which the main concern revolves around the toxicological and environmental impact of a specific solvent more than the overall consumption, searching for an alternative, green solvent can be an option. However, this is often associated with vigorous testing and adaption of the analytical method to ensure a constant performance. Moreover, solvents that are promising as replacements in synthesis cannot always be applied in LC, as the technology is oftentimes unsuitable for high viscosity solvents due to instrumental limits in backpressure [2–4].

Chromatographic miniaturisation is the most obvious approach to reduce overall solvent consumption. By decreasing the column inner diameter and consequently the flow rate, many users believe to

effortlessly adapt a method to drastically cut the solvent amount. This is oftentimes advertised in micro- and nano-LC applications, as well as in ultra high-performance liquid chromatography. However, this also comes with challenges of analytical performance and robustness [2,5]. Furthermore, when considering the solvent savings solely on the basis of mobile phase consumed, it can disregard the potential for further improvement during other stages within the analytical process. This means that depending on sample preparation method, required dilution of the samples, and calibration standards, as well as number of samples, it can be much more economical to miniaturise the sample preparation process than the analysis itself.

However, when reducing the amount of solvent used in sample preparation, the result becomes susceptible to inaccuracies. The requirement of high dilutions by factors of  $10^3$  to  $10^6$  in a multi-step process demands for a dilution process with high accuracy for every step. In order to minimise the systematic error, the most common way for sample dilution is employment of (relatively spoken) high-volume glassware and pipettes. Simply using smaller glassware is rarely possible without increasing the instrument error of the method. The

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same is true for other low-volume methods, which employ adjustable dispensers such as piston pipettes or syringes. This is only tolerable in cases where the volumetric uncertainty is smaller than the difference between values that are relevant for the analytical question, and subsequent decision. For trace level analyses, the higher uncertainty of low-volume instrumentation mathematically affects the result more than side components with low-percentage amounts. However, the dilution error is not decisive in many trace level analyses, as the tolerance for deviation is usually higher for lower values. This is not the case for e.g. pharmaceutical, agricultural, and nutritional side components, where this deviation can be critical for product release.

Volumetric methods also come with the downside of being temperature dependent. When working in a laboratory with no or insufficient air conditioning, density of the solvents, and consequently the results, might vary between analyses. Another issue when working with instruments with adjustable volumes is that they are only required to be calibrated for one solvent, usually deionised water [6]. When working with differing volumes, the results get increasingly inaccurate for other solvents and mixtures, especially at inconsistent temperature conditions. However, LC often requires working with organic solvents, and samples are regularly diluted with more than one solvent, namely one for sample dissolution and one for injection. Also, when considering automation approaches, differences in viscosity might require adaption of draw speeds for each method to exclude the possibility of large air bubbles, making them less versatile.

One potential approach is switching from a volumetric to a gravimetric dilution of samples and standards. Gravimetric sample preparation is based on the mass fraction of an analyte in a given solvent. A notable advantage is the ease of retaining accuracy even after a user error has occurred. Variations in sample or solvent mass can be corrected either by direct adaption of the required diluent, or by calculating the actual final mass fraction, regardless of the final solvent amount. The instrument error is restricted to that of the analytical balance which is easy to determine and comparatively low. It also remains constant for all volumes and at all temperatures, as long as the minimal weight is respected. For many analytical balances, this is <20 mg [7], meaning less than two droplets of most liquids. Overall, this approach is easy to adopt and implement, as no further instrumentation apart from an analytical balance are necessary. Automation is also possible with the help of robotic liquid handling workstations, as the one depicted in Fig. 1.

Overall, this study is an introduction to gravimetric and automated methods for sample preparation in LC. The aim is to demonstrate the advantages of these techniques on an environmental, economic, and technological level. In the context of sample preparation, discussing the environmental benefits of “greenness” of a workflow needs to be evaluated with a different focus than the subsequent analysis. Hence, ten principles of green sample preparation have been introduced [8] and a tool named AGREeprep was designed to reflect these advantages in an accessible way [9]. Further observations such as performance and monetary or social aspects are to be discussed as well. However, the first step is to elaborate on the idea and theory of gravimetric dilution approaches.

### 1.1. Theory and calculation

As most volumetric methods rely on volumetric concentrations (e.g. mass concentration or molar concentration), a conversion needs to be performed in cases in which the established volumetric method is modified into a gravimetric one. To convert the concentration into a mass fraction, a trial dilution can be performed where the volumetric dilutions are weighed after every step and the actual mass fraction is determined. An easier alternative is to estimate the mass fraction via the density of the solvent, see Eq. (1).

$$w_{\text{target}} = \frac{m_{\text{sample, target}}}{V \times \rho_{\text{diluent}}} \quad (1)$$

in which  $w_{\text{target}}$  is the target mass fraction in mg/mg,  $m_{\text{sample, target}}$  is the target weight of the sample or standard substance to be dissolved/diluted in mg,  $V$  is the volume of the diluent in volumetric dilution steps in mL, and  $\rho_{\text{diluent}}$  is the density of the diluent in mg/mL.

The exact final mass fraction can be determined via sample and total weight, also accounting for the purity of the sample or standard substance, which is why conversion by density is usually sufficient. It is, however, not recommended to re-convert the calculated mass fraction into a concentration via density, as results are not accurate enough in most cases.

Once the initial sample weight has been determined, the target total weight can be calculated according to Eq. (2).

$$m_{\text{total, target}} = \frac{m_{\text{sample}}}{w_{\text{target}}} \quad (2)$$

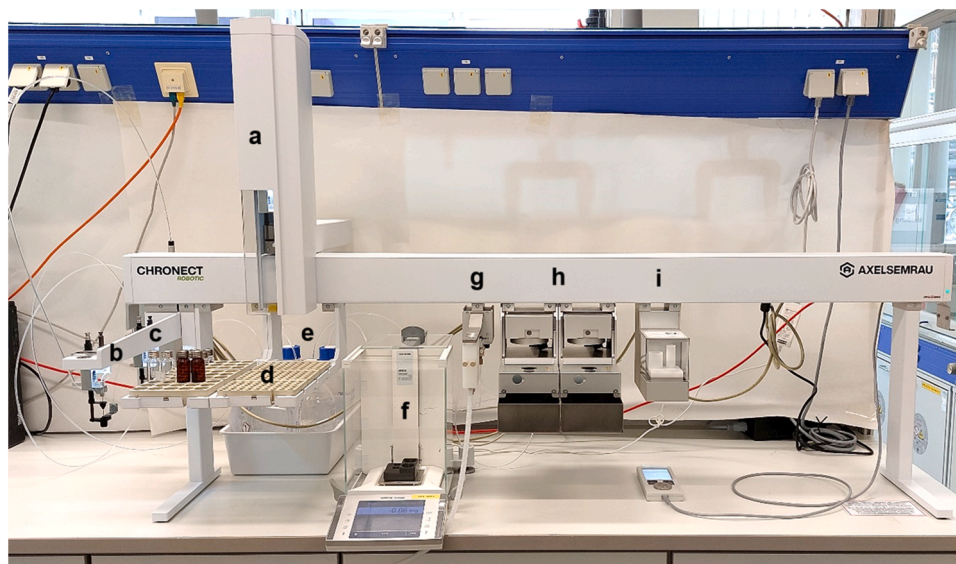


Fig. 1. PAL3 automated liquid handling station by Axel Semrau, equipped with a) robot arm, b) dilutor tool (connected to the solvent reservoir), c) syringe tools, d) sample rack, e) solvent reservoir, f) analytical balance, g) rinsing station, h) decapping station, i) vortexer.

in which  $m_{\text{total,target}}$  is the desired total weight after dilution in mg,  $m_{\text{sample}}$  is the weight of the sample in mg, and  $w_{\text{target}}$  is the desired mass fraction of the solution in mg/mg.

When working with automated liquid handling stations, they are unable to translate masses on a scale into the amount of solvent dispensed via their syringe and dilutor. Thus, the volume of the sample (in solution) needs to serve as basis for the dilutor volume. In the dilution step, the dispensed volume of the dilutor is calculated based on the sample weight, the estimated diluent density, and the desired dilution factor with Eq. (3). Subtraction of the sample weight ("1") from the dilution factor is necessary as not to include the sample weight twice within the calculation.

$$V_{\text{dilutor}} = \frac{m_{\text{sample}} \times (F - 1)}{\rho_{\text{diluent}}} \quad (3)$$

in which  $V_{\text{dilutor}}$  is the volume dispensed by the dilutor in mL,  $m_{\text{sample}}$  is the weight of the substance to be diluted (previously added solution) in mg,  $F$  is the dilution factor, and  $\rho$  is the estimated density of the sample diluent in mg/mL.

Finally, the uncertainties of the sample preparation instrumentation are calculated for the manual volumetric as well as the gravimetric methods. Balance uncertainty is calculated on the basis of a Gauss distribution (Eq. (4)), uncertainties of pipettes and flasks on the basis of a rectangular distribution (Eq. (5)). The combined uncertainty for the entire dilution series was then calculated according to Eq. (6) [10]

$$u = \frac{a}{k} \quad (4)$$

with  $u$  being the standard deviation,  $a$  referring to the instrument uncertainty value, and  $k$  representing the coverage factor for a certain level of confidence  $p$  (here:  $p = 68.27\%$ ,  $k = 1$ ).

$$u = \frac{a}{\sqrt{3}} \quad (5)$$

with  $u$  being the standard deviation and  $a$  referring to the instrument uncertainty value.

$$u_c(y) = \sqrt{\sum_{i=1}^n [u_i(x_i)]^2} \quad (6)$$

with  $u_c(y)$  being the uncertainty of  $y$  (combined uncertainty) and  $u_i(x_i)$  being the uncertainty of  $x$  (components).

## 2. Materials and methods

### 2.1. Materials

#### 2.1.1. Chemicals

The solvents used in this study were ultrapure water, purified by a Millipore Milli-Q Integral 3 water purifier from Merck (Darmstadt, Germany), acetonitrile,  $\geq 99.9\%$  from Honeywell (Charlotte, NC, USA), methanol,  $\geq 99.8\%$  from Supelco, Merck, and tetrahydrofuran (THF),  $\geq 99.9\%$  from Honeywell. Phosphoric acid, 85 wt-% in H<sub>2</sub>O from Sigma-Aldrich (St. Louis, MO, USA) served as an additive for pH regulation.

The analytes for instrument qualification were methylparaben (MP),  $\geq 99.0\%$ , ethylparaben (EP), 99%, propylparaben (PP),  $\geq 99\%$ , and butylparaben (BP),  $\geq 99.0\%$ , all from Sigma-Aldrich. For dilution method comparison, phenothiazine, 100% and maleic acid, 99.7% reference substances were employed. Additionally, the samples analysed for phenothiazine and maleic acid respectively were provided by BASF (Ludwigshafen, Germany).

#### 2.1.2. Instrumentation

The following section will present the instruments utilised in this study, as well as their systematic errors. For volumetric sample

preparation, the following glassware and pipettes were used. All listed instrument errors are measured at 20 °C.

Glass pipettes from Hirschmann Laborgeräte (Eberstadt, Germany):

- 0.5 mL  $\pm$  0.005 mL
- 1 mL  $\pm$  0.008 mL
- 2 mL  $\pm$  0.01 mL
- 3 mL  $\pm$  0.01 mL
- 4 mL  $\pm$  0.023 mL
- 5 mL  $\pm$  0.015 mL

Volumetric flasks from witeg Labortechnik (Wertheim, Germany):

- 5 mL  $\pm$  0.04 mL
- 25 mL  $\pm$  0.060 mL
- 50 mL  $\pm$  0.100 mL
- 100 mL  $\pm$  0.100 mL

The analytical balance for manual gravimetric sample preparation was a XPR225 DeltaRange (max. 121 g / 220 g,  $d = 0.01$  mg / 0.1 mg) from Mettler-Toledo (Greifensee, Switzerland) which is calibrated for a minimum sample weight of 20 mg.

Microman positive displacement piston pipettes by Gilson (Middletown, WI, USA) were used, namely the models M100E (10  $\mu$ L  $\pm$  0.50  $\mu$ L to 100  $\mu$ L  $\pm$  1.00  $\mu$ L) and M1000E (100  $\mu$ L  $\pm$  3.0  $\mu$ L to 1000  $\mu$ L  $\pm$  8.0  $\mu$ L). For volumes  $> 1$  mL, a HandyStep touch device for repetitive pipetting by Brand (Wertheim, Germany) with a 10 mL  $\pm$  0.4 mL tip was used.

The automation was facilitated by a PAL3 robotic tool change (RTC) system from CTC Analytics (Zwingen, Switzerland), as equipped by Axel Semrau (Sprockhövel, Germany) with a 5 mL dilutor tool and a 100  $\mu$ L syringe tool and an additional XPR205 DeltaRange analytical balance (max. 121 g / 220 g,  $d = 0.01$  mg / 0.1 mg), also from Mettler-Toledo. Method programming was done with the Chronos software by Axel Semrau. The liquid handling system was a loan instrument and was situated and tested in an industrial laboratory at BASF SE in Ludwigshafen for a period of six months. All analyses were performed during this time.

For LC analysis, two 1290 Infinity II model LCs from Agilent Technologies (Waldbronn, Germany) are used. They consist of a G7120A High Speed Pump, a G7167B 1290 Multisampler, and a G7116B 1290 MCT column compartment each, as well as either a G7117B 1290 diode array detector (DAD) or a G4212A DAD. Chromeleon 7.2 by Thermo Fisher Scientific (Waltham, MA, USA) was used for instrument operation and analysis. Further analysis and visualisation were performed using Origin 2024 by Origin (Northampton, MA, USA) and Microsoft Excel, Version 2408 (Redmond, WA, USA).

### 2.2. Methods

The density was roughly estimated by weighing 5 mL (volumetric flask) of the prepared solvent at 23 °C (room temperature). This generally yielded elevated density values and consequently lower dilution factors in gravimetric approaches, but did not affect the results in a significant way.

Calibration solutions were prepared in the following five ways:

A) Manual high-volume volumetric sample preparation (HVSP)

The reference substances are dissolved and diluted according to standard laboratory practice. Glassware includes glass pipettes for volumes of 0.5 to 4 mL as well as volumetric flasks of 25 to 50 mL. As this is the most established and validated method in our laboratories, it is used as the reference method.

B) Manual low-volume volumetric sample preparation (LVSP)

The procedure is analogous to the manual high-volume sample preparation. However, glass pipettes and volumetric flasks are replaced by pipetting devices with adjustable volumes in the range of 10  $\mu\text{L}$  to 10 mL. The overall solvent volume is reduced by a factor of 2.5 (intermediate dilutions) to 25 (calibration solutions).

### C) Manual gravimetric sample preparation (MGSP)

Based on the mass fraction of the volumetric method's standards, the required amounts of sample and solvent are estimated with the equations described in Section 1.1. Weighing takes place after addition of the reference substance/stock solutions and the solvent respectively.

### D) Automated volumetric sample preparation (AVSP)

The RTC PAL is programmed to simulate volumetric dilution of the stock solutions utilizing syringes and the dilutor tool in the range of approximately 20  $\mu\text{L}$  to several mL.

### E) Automated gravimetric sample preparation (AGSP)

Similar to the automated volumetric method, the syringe and dilutor tool perform the sample preparation. Weighing steps of the containers are before and after each liquid transfer. The equation can be found in Section 1.1. A short video demonstrating the process can be found in Appendix B.

Dilution factors for calibration according to methods A) and B) can differ from C), D) and E), when the aim is to space out the calibration points as evenly as possible. This is easily possible for gravimetric as well as automated, computer-programmed methods. When relying on quantitated dilution tools, such as pipettes, an alternative dilution factor in the same range must be chosen. Some PAL methods involve repeated syringe dispensing steps to ensure a minimum basis weight of 20 mg.

To qualify the liquid handling station, two parameters are investigated: reproducibility and carryover. Repeatability of mixing and diluting is investigated via relative standard deviations (RSD) of peak areas, peak area ratios, and response factors of the paraben stock solutions (double determination and comparison to HVSP). Carryover is evaluated for the sample-handling syringe and the dilutor needle, by checking cross-contamination and solvent blanks.

The phenothiazine calibration consists of two stock solutions with approximately 25 mg (stock solution 1) and 45 mg (stock solution 2) of the reference standard dissolved in 50 mL of the diluent (Table 3), each. The weights are recorded, and the stock solutions serve as basis for all following dilution approaches. Table 1 presents the target dilution factors, while detailed information on dilution steps and glassware is listed in the supporting information in Table S1. The preparation procedure of the sample is also described there.

The maleic acid calibration consists of two stock solutions with approximately 30 mg (stock solution 1) and 50 mg (stock solution 2) of the reference standard dissolved in 50 mL of the diluent (Table 3), each.

**Table 1**

Target dilution factors for the phenothiazine calibration series of each dilution method. All dilutions are based on the same two stock solutions.

Level	Target dilution factor for method				
	HVSP	LVSP	MGSP	AVSP	AGSP
Intermediate from stock solution 1	25	25	25	25	25
Intermediate from stock solution 2	25	25	25	25	25
Calibration level 1*	25	25	25	25	25
Calibration level 2 <sup>†</sup>	25	25	19.3	19.3	19.3
Calibration level 3*	8.3	8.3	6.8	6.8	6.8
Calibration level 4 <sup>†</sup>	8.3	8.3	9	9	9

\* From intermediate 1.

<sup>†</sup> From intermediate 2.

The weights are recorded, and the stock solutions serve as basis for all following dilution approaches. Additionally, instrument errors for a dilution series are calculated for calibration levels 1 and 2 for the HVSP and MGSP methods. Table 2 presents the target dilution factors, while detailed information on dilution steps and glassware is listed in the supporting information in Table S2. The preparation procedure of the sample is also described there.

Phenothiazine and maleic acid samples are real samples sent to the laboratory by customers and were analysed several days before this study.

The separation conditions for the aforementioned samples are listed in Table 3 below.

The accepted limit for deviations is set to 5 % for all presented analyses which corresponds to the general instrument error of the LC method.

Finally, the AGREeprep tool is applied to maleic acid. All dilution methods are compared for the lowest calibration level. Each of the metric's criteria can be weighted freely depending on the user's requirements [9]. In this study, the default weighting was chosen, as it is most prominent in literature and thus most widely comparable.

## 3. Results and discussion

In order to properly discuss the results of this study, it has to be noted that the PAL3 liquid handling station used for the automated methods was only accessible as a loan instrument for a limited time. The focus of this study hence did not lie on the validation of the individual methods, but on the qualification of the PAL3 instrument in Section 3.1, as well as some exemplary feasibility studies, two of which are presented in Section 3.2. Due to the time restriction in our exploration of automated methods, this study shall serve as a first-impression report and as inspiration to other users instead of the description of a fully validated workflow.

### 3.1. PAL instrument qualification

Overall, the instrument qualification of the liquid handling robot proved to be successful. Although not explicitly tested, throughout the study many different volumes were diluted to various degrees. Even for very high dilutions, the results remained reliable as long as a minimum of 20 mg were available for weighing. For the most part, dilution factors were roughly between 3 and 200.

In the separation of the paraben mix, depicted in Fig. 2, deviations in response factors and peak area ratios were all around 1 % (and around 2 % for propylparaben, which is discussed below) across both manual volumetric and automated gravimetric preparation approaches. As the recorded PAL dilution factors differed slightly from the target factors achieved in the volumetric dilution series, the peak areas could not be compared directly. They were corrected to "undiluted" peak areas by multiplication via the dilution factor. While the relative standard deviations were all calculated to be 2 % at maximum, the evaluation of the

**Table 2**

Target dilution factors for the maleic acid calibration series of each dilution method. All dilutions are based on the same two stock solutions.

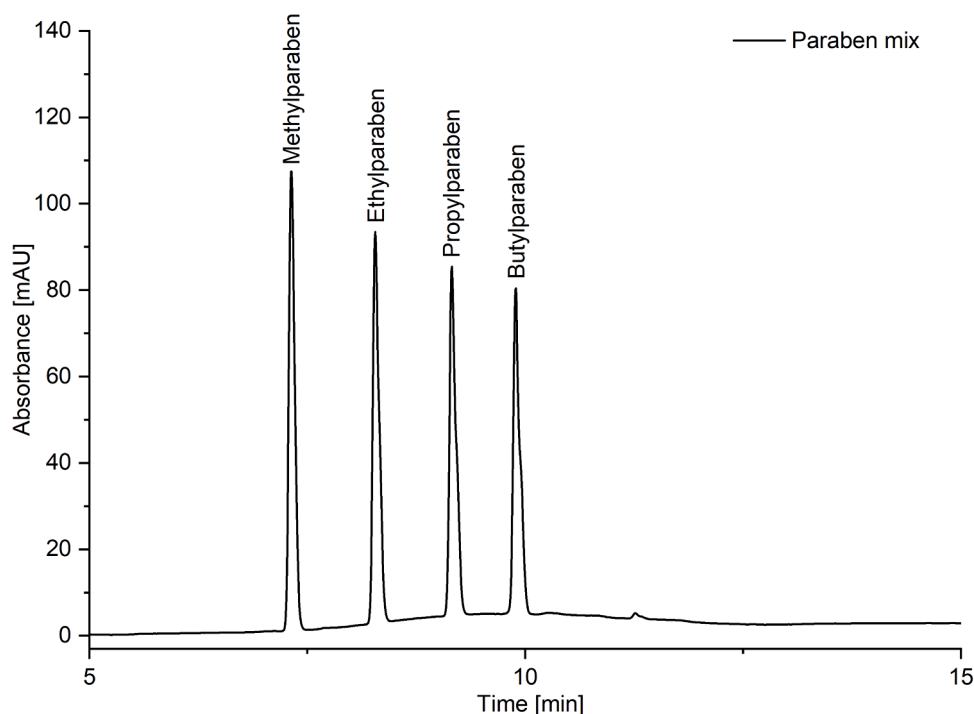
Level	Target dilution factor for method				
	HVSP	LVSP	MGSP	AVSP	AGSP
Intermediate from stock solution 1	25	25	25	25	25
Intermediate from stock solution 2	25	25	25	25	25
Calibration level 1*	50	50	50	50	50
Calibration level 2 <sup>†</sup>	12.5	12.5	12.5	12.5	12.5
Calibration level 3*	12.5	12.5	12.5	12.5	12.5
Calibration level 4 <sup>†</sup>	6.25	6.25	10	6.25	6.25

\* From intermediate 2.

<sup>†</sup> From intermediate 1.

**Table 3**  
Chromatographic conditions.

Method	Parabens	Phenothiazine	Maleic acid
Sample diluent	1:1 acetonitrile/0.1 % phosphoric acid (v/v)	1:1 acetonitrile/0.1 % phosphoric acid (v/v)	1:9 acetonitrile/0.1 % phosphoric acid (v/v)
Column	Halo Phenyl-Hexyl, 2.7 $\mu$ m, 3.0 $\times$ 150 mm	Polaris 3 C18, 3 $\mu$ m, 4.6 $\times$ 150 mm	Halo Phenyl-Hexyl, 2.7 $\mu$ m, 3.0 $\times$ 150 mm
Column temperature	25 $^{\circ}$ C	25 $^{\circ}$ C	25 $^{\circ}$ C
Flow	0.7 mL/min	1.0 mL/min	0.7 mL/min
Injection volume	5.0 $\mu$ L	10.0 $\mu$ L	10.0 $\mu$ L
Mobile phase	A: 0.1 % phosphoric acid B: 980:20 methanol/water (v/v)	A: 70:30 0.1 % phosphoric acid/acetonitrile (v/v) B: 70:27:1.5:1.5 acetonitrile/methyl tert-butyl ether/water/ 2-propanol (v/v/v/v)	A: 0.1 % phosphoric acid B: 980:20:1 methanol/water/ phosphoric acid (v/v/v)
Gradient	0 min: 0 %B – 12 min: 100 %B – 15 min: 100 %B – 16.5 min: 0 %B	0 min: 0 %B – 10 min: 100 %B – 10.5 min: 100 %B – 15 min: 0 %B	0 min: 0 %B – 12 min: 100 %B – 15 min: 100 %B – 16.5 min: 0 %B
UV detection wavelength	257 nm	252 nm	205 nm
Run time	20 min	25 min	20 min



**Fig. 2.** Chromatogram of the paraben mix used for PAL instrument qualification.

response factors seemed more sensible.

All relative standard deviations were within the desired range of 5 %, as is depicted in Table 4, with larger deviations being found for calculations involving the propylparaben signal. However, this is not thought to be a result of the automated dilution, as manual approaches for the preparation of this sample mixture exhibit the same behaviour. It is instead believed to be an effect of the digital peak evaluation method, as the propylparaben signal was situated on a bump on the base line that made it difficult to integrate.

As for the carryover, both the investigation of the sample-carrying syringe as well as the dilutor did not hint to a carryover issue within the system. No traces of previously handled substances could be found in subsequently prepared samples, as is demonstrated in Fig. 3.

For the duration of the study, the carryover was monitored for samples with more complex matrices. However, even then, no issues were ever detected (data not reported).

### 3.2. Application

In the following section, two applications with industrial relevance shall be discussed. Phenothiazine as an additive is often used as stabiliser and inhibitor in oxidation-sensitive environments during and after synthesis, especially in acrylates [11]. Maleic acid, aside from being a raw material for many industrial products such as water-soluble polymers [12], moreover finds use as an additive, for example as adhesion promoter [13].

One representative analysis for each analyte is presented below. The result for the same (set of) signal(s) was calculated with five different evaluation methods, one for each calibration set. The manual high-volume volumetric sample preparation was used as the reference, as it is the current laboratory standard and known to be sufficiently accurate on the required concentration level. The results are presented in Tables 5 and 6 below. Here, the total solvent volume represents the solvent consumed for dilution of the calibration stock solutions. The numbers in brackets denote the total solvent volume including the stock solutions, which were the same for all methods. In the case of separate stock

**Table 4**

Peak areas, response factors, and peak area ratios for the methylparaben signal mixed and diluted volumetrically as well as gravimetrically. Actual gravimetric dilution factors are also given.

Dilution	Methylparaben					
	Amount [mg/mL or mg/mg]	Peak area [mAU <sup>2</sup> min]	Response factor	Ratio EP/MP	Ratio PP/MP	Ratio BP/MP
HVSP 20x	0.0118	8.37	667.00	0.90	0.84	0.80
	0.0118	8.36	666.05	0.90	0.85	0.80
HVSP 10x	0.0236	16.69	664.88	0.90	0.86	0.80
	0.0236	16.71	665.49	0.90	0.84	0.80
HVSP 4x	0.0590	41.67	663.86	0.92	0.86	0.80
	0.0590	41.56	662.11	0.92	0.86	0.80
AGSP 19.4x	0.0130	8.47	652.44	0.90	0.84	0.79
	0.0130	8.46	651.80	0.90	0.84	0.81
AGSP 9.8x	0.0257	17.18	667.57	0.90	0.88	0.79
	0.0257	16.99	660.54	0.90	0.84	0.79
AGSP 4.0x	0.0631	41.82	662.77	0.91	0.85	0.79
	0.0631	41.79	662.27	0.91	0.85	0.79
		Mean	662.23	0.91	0.85	0.80
		Standard deviation	5.17	0.01	0.01	0.01
		RSD [%]	<b>0.78</b>	<b>0.76</b>	<b>1.54</b>	<b>0.92</b>

Legend: HVSP manual high volume sample preparation AGSP automated gravimetric sample preparation.

MP methylparaben EP ethylparaben.

PP propylparaben BP butylparaben.

RSD relative standard deviation.

solutions for all methods, the solvent consumption for the gravimetric solutions is expected to be even lower, as they can be further reduced when reducing the initial weight of the reference substance to the minimum weight of the analytical balance.

For both examples, the automated volumetric sample preparation shows the highest deviations. All PAL dilution methods are programmed to be as universally applicable as possible. For volumetric PAL methods, this is a disadvantage, as the draw speed of the syringe is not adjusted to the viscosity of the dilution and sample solvents. This can lead to an increase of air drawn by the syringe. In gravimetric PAL methods, the actual amount of sample that is transferred and diluted is known by weight, which can shift the calibration point but also increases accuracy.

Thus, the gravimetric PAL methods show the least deviation to the volumetric reference method.

While this should also be the case for the manual gravimetric method, the results here demonstrate the dependence on the appropriate method on the dilution solvent. For the maleic acid, a mostly aqueous solvent is used. This both means that the manual low-volume volumetric method exhibits the best fitted calibration function of all methods, as well as better results than the manual gravimetric sample preparation method, unlike the phenothiazine sample. One reason is that the low-volume pipetting instruments and disposable tips are usually calibrated for water. The second one is the relative difficulty of manually diluting with water-rich solvents, as water tends to be difficult to handle due to high surface tension and low volatility, especially compared to some organic solvents used in LC. This leads to water droplets sticking to the joint of the flask, slowly evaporating around the glass stopper. Consequently, a steady weighing process is complicated and the deviation of the result increases. Even then, the manual gravimetric sample preparation shows very satisfying results and is thus more universally applicable than the manual low-volume volumetric process. Regarding the solvent usage, the gravimetric approaches unite that with a drastically reduced solvent amount, as well as a valid result.

Additionally, HVSP was performed by one other operator in the

**Table 5**

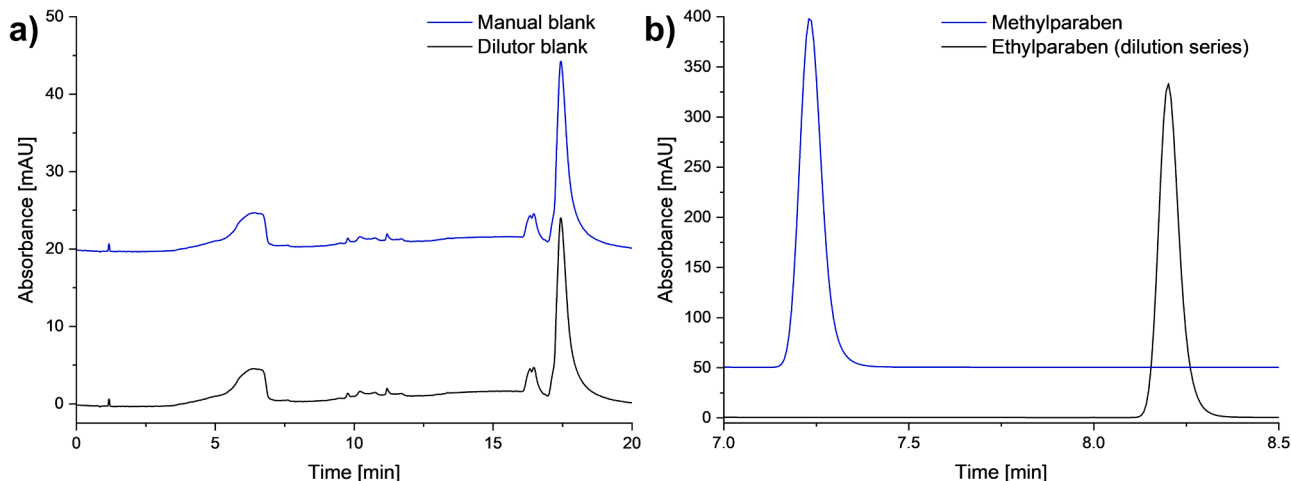
Results for phenothiazine. The numbers in brackets denote the total solvent volume including the stock solutions.

Dilution method	HVSP	LVSP	MGSP	AVSP	AGSP
Result [ppm]	210.99	216.19	210.04	197.70	212.79
Deviation from HVSP [%]	0	2.46	-0.45	-6.30	0.85
R <sup>2</sup> of calibration	0.99988	0.99992	1	0.9169	0.99989
Total solvent volume [mL]	140 (240)	23 (123)	19 (119)	13 (113)	11 (111)

**Table 6**

Results for maleic acid. The numbers in brackets denote the total solvent volume including the stock solutions.

Dilution method	HVSP	LVSP	MGSP	AVSP	AGSP
Mean result [ppm]	556.76	561.39	565.21	595.88	558.91
Deviation from HVSP [%]	0	0.83	1.52	7.03	0.39
R <sup>2</sup> of calibration	0.99984	0.99999	0.99859	0.99972	0.99989
Total solvent volume [mL]	139.5 (239.5)	23 (123)	18 (118)	13 (113)	10 (110)



**Fig. 3.** Chromatograms of the investigations into carryover effects for a) the dilutor tool and b) the sample syringe.

course of answering the underlying customer request. However, due to the fast consumption of the analyte, the results of the phenothiazine analyses, which were performed several days apart, were not comparable. For the maleic acid sample, deviation between both HVSP-based results were found to be 1.3 %, further demonstrating the impact that a manual sample preparation can have on the result.

In these examples, even when the volume of the stock solutions is higher than necessary for the gravimetric approaches, the solvent savings are in the realm of 90 % or more along the various calibration series when compared to HVSP. This percentage is also reflected on the monetary saving of the method. For the presented examples, they are estimated to be 95 € for the phenothiazine calibration and 40 € for the maleic acid calibration series [14]. Still, it is impossible to generalise statements about solvent savings, as they heavily depend on the type and number of samples and reference points in any given analysis.

However, additional costs caused by automation need to be considered as well. Assuming two calibration series per workday such as the ones describes above, and a total cost of approximately 60k € for the presented PAL setup (without analytical balance which was already present in the laboratory), amortisation would take 800 calibration series, or just over one year. As the actual energy consumption of the liquid handling station was not measured, operating costs are determined via the maximum power listed in the PAL user manual [15]. With an occupancy of two dilution series per day, equal to about two hours, additional energy costs would lie below 50 € per year. Even with continuous use at full power throughout the entire year, energy costs would amount to approximately 500 € per year (industrial prices for Europe, approximately 0.2 €/kWh) [16]. This usage would in turn shorten amortisation time, thus, energy costs are considered to be negligible.

The largest (calibration level 1 of maleic acid) and lowest (calibration level 2 of maleic acid) instrument errors for an HVSP dilution series were calculated to be 0.008 % and 0.006 % uncertainty respectively. For gravimetric methods, a “worst case” scenario was calculated in which the lowest weighable value is assumed. It resulted of a maximum uncertainty of 0.001 % [10]. These results further demonstrate the impact of the volumetric methods on the estimation of the true value. As mentioned in Section 1, it is therefore important to consider in which cases this uncertainty can be tolerated and reveals one more advantage of gravimetric approaches.

When applying the AGREEprep metric to the lowest level of the maleic acid calibration series, it becomes apparent that the HVSP method is the least green (Fig. 4a), scoring lowest due to the high solvent consumption. The LVSP approach (Fig. 4b) can drastically reduce the amount of solvent, and thus hazardous materials, used. Still, the final score is the second lowest of the group, as it is the only method that requires single-use plastic pipette tips, which increases waste. All other methods rely on reusable glassware. Next, the score for the MGSP (Fig. 4c) ranges just in the middle of manual volumetric and automated methods. While the solvent consumption is much improved compared to HVSP and closer to that of all other methods, and it requires less energy than automation, manually preparing gravimetric samples is a laborious task that affects the sample throughput in a negative way and exposes the operator to additional hazards. However, due to the convincing

performance and easy implementation, it can be a method worth considering, especially if the task can be digitally supported by direct transfer of the weighed value to template sheets. Meanwhile, AVSP (Fig. 4d) yields the highest score, as it has among the lowest sample and waste volumes and the highest sample throughput. However, this method has already been found to be lacking in performance, unless it is a very specific method programmed for a routine sample. Then, solvent viscosity can be accounted for, and the exact dispensed volumes can be verified. Finally, the AGSP approach (Fig. 4e) only has a slightly lower score. The main factors here are the reduced number of steps, an increase in automation, and a decrease in hazardous substances and other occupational dangers. Most prominently, solvent use and overall waste mass are much lower than for HVSP, as was to be expected. Contrarily, and although it does not affect the outcome of the comparison, the energy consumption of the automated methods, i.e. the liquid handling station, is higher than that of manual methods. However, as is also pointed out in the original AGREEprep article [9], operation manuals [7, 15, 17] of electrical devices oftentimes give the maximum value for energy consumption although the devices rarely operate at maximum power. This means the required power is likely overestimated, although this was not measured in the course of this study.

For the here presented evaluation, only the volume of the final dilution step was considered, as stock and intermediate solutions can be reused for other calibration levels as well. Generally, the AGREEprep metric is not thought to be suitable here, as it and its scoring limits are designed to calculate the greenness of a single sample. In the case of dilution series in which there are great variations for each level, the comparison of one sample at a time and the resulting evaluation limits are not considered to yield a fair comparison. Meanwhile, comparison of the whole series is impossible without overshooting the limits for each parameter, resulting in a misrepresentation of the actual greenness. The here presented result is considered to be a compromise that also unveils the disadvantages of the AGREEprep metric for such applications.

Finally, the social impact on the automation of sample preparation can be considered. While parameters such as mental health and contentedness of lab personnel are hard to quantify within the scope of a chemical study such as this, the following notes are based on conversations with lab technicians who performed the same procedures in parallel. With a decline of trained laboratory personnel and a rising demand in fast and reliable analyses, even the option to manually prepare the samples while the calibration solutions are simultaneously prepared on an automated system is a notable relief for lab technicians. Moreover, some protocols demand multiple steps performed with precise timing, which the sample preparation robot can do more reliably, while making the sample preparation process less stress-inducing for the operator.

Although only two examples are presented here, throughout the loan period, several other sample preparation methods for various samples and analytes with different demands concerning solvents, mixing ratios, and viscosity were performed. During this time, single-step dilutions with factors of 2.5 to 275 were programmed, even though the best results were achieved with a minimum stock solution weight of 20 mg, which corresponds to a dilution factor of approximately 50 if directly diluted into a 1.5 mL vial. However, multi-step dilutions were also easy

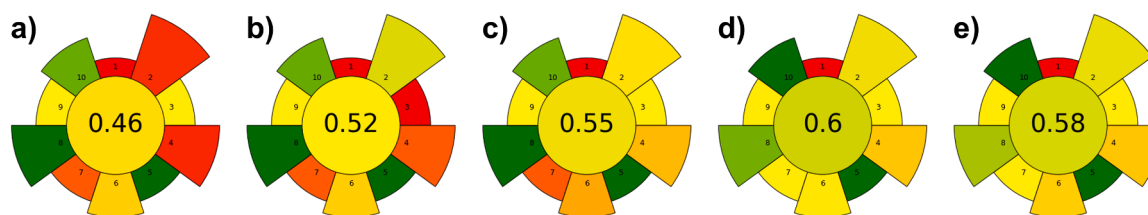


Fig. 4. AGREEprep scores of calibration level 1 of the maleic acid sample preparation method for a) high-volume sample preparation, b) low-volume sample preparation, c) manual gravimetric sample preparation, d) automated volumetric sample preparation, e) automated gravimetric sample preparation.

to implement and automate, thus, final concentrations down to 0.05 mg/kg could be analysed (data not reported). There were no instances in which performance was not at least equal, while solvent consumption was drastically reduced. As this study was performed in an industrial setting, comparative measurements and validation efforts such as manual dilution by different operators were not possible to perform, ironically due to the high demand of customer requests, and a consequent lack of time.

#### 4. Summary and outlook

This study shows that it is possible to adapt sample preparation in LC in such a way that 90 % of the required solvent can be cut, leading to substantial economic and environmental benefits. When employing gravimetric dilution approaches, this improvement can be achieved reliably, reproducibly, and is widely applicable for all solvents, regardless of solvent viscosity, density, or temperatures without any adaptations. Automation of gravimetric methods allow for the programming of methods which are more flexible in settings with changing conditions as automation of volumetric methods, as dilution factor correcting is inherent to the calculation of the resulting mass fractions. This saves time and effort, and requires less user knowledge, compared to optimisation of automation of a volumetric method. While low-volume volumetric approaches, i.e. by reducing the size of the pipette, also lead to a reduction in solvent consumption, they also show less consistent results and a higher instrument error. Especially when comparing automated methods, the gravimetric approach united accurate results with many more benefits.

Aside from ecological, economic and technological improvements, there also is a social component to automation. It grants social sustainability in terms of stress-reduction for complex or time-consuming manual (gravimetric) methods, while improving method repeatability, making it independent of user handling, as well as overall performance and productivity. The presence of a sample preparation robot allows preparation of sample and calibration standards in parallel, which could lead to a higher sample throughput.

Due to declining numbers of trained personnel in laboratories and expectations of faster and more reliable analyses, especially sample preparation labs will have to rely on versatile robotic stations in the future. In the context of LC, this also means full automation, employing robots which can collect, transport, weigh, extract, dilute, derivatise, and inject the sample. The final steps of computerised data evaluation and decision-making create an independently operating laboratory space even for non-routine analyses.

#### CRedit authorship contribution statement

**Mo Legelli:** Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Marcel Vranceanu:** Methodology, Conceptualization. **Michaela Wirtz:** Supervision. **Stefan Lamotte:** Writing – review & editing, Supervision, Project administration, Funding acquisition,

Conceptualization.

#### Declaration of competing interest

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#### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.greeac.2025.100279](https://doi.org/10.1016/j.greeac.2025.100279).

#### Data availability

The authors do not have permission to share data.

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