Keeping the Workflow in Focus

Practical implementation of a gravimetric approach to sample preparation

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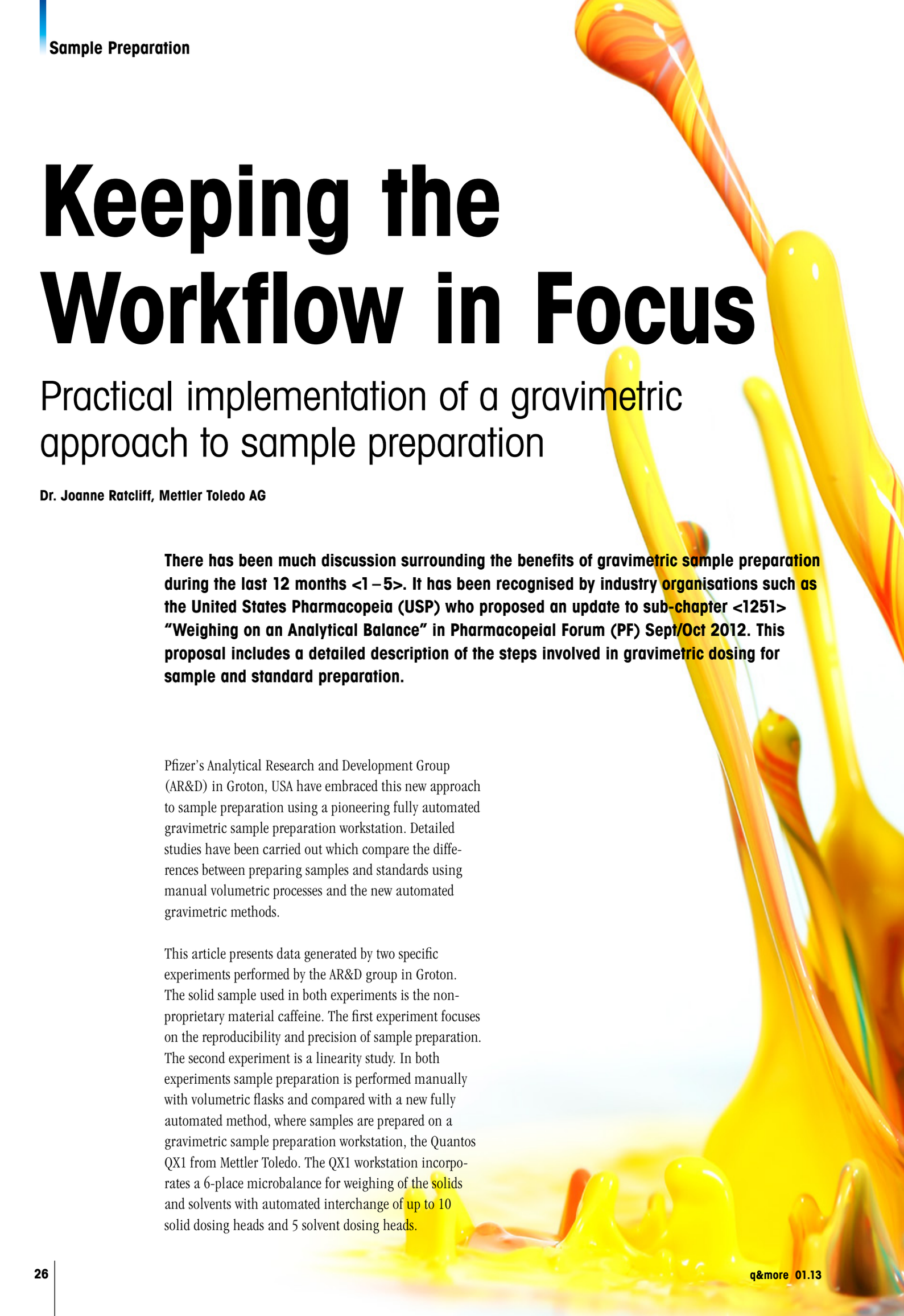

There has been much discussion surrounding the benefits of gravimetric sample preparation during the last 12 months <1 – 5>. It has been recognised by industry organisations such as the United States Pharmacopeia (USP) who proposed an update to sub-chapter <1251> "Weighing on an Analytical Balance" in Pharmacopeial Forum (PF) Sept/Oct 2012. This proposal includes a detailed description of the steps involved in gravimetric dosing for sample and standard preparation.

Pfizer's Analytical Research and Development Group (AR&D) in Groton, USA have embraced this new approach to sample preparation using a pioneering fully automated gravimetric sample preparation workstation. Detailed studies have been carried out which compare the differences between preparing samples and standards using manual volumetric processes and the new automated gravimetric methods.

This article presents data generated by two specific experiments performed by the AR&D group in Groton. The solid sample used in both experiments is the non-proprietary material caffeine. The first experiment focuses on the reproducibility and precision of sample preparation. The second experiment is a linearity study. In both experiments sample preparation is performed manually with volumetric flasks and compared with a new fully automated method, where samples are prepared on a gravimetric sample preparation workstation, the Quantos QX1 from Mettler Toledo. The QX1 workstation incorporates a 6-place microbalance for weighing of the solids and solvents with automated interchange of up to 10 solid dosing heads and 5 solvent dosing heads.

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Sample Set Name: MW Manual Repeatability
Sample Set Id: 18622 Result Set Id: 18740

	Sample Name	Result ID	Name	Area (µV*sec)	Potency 1 (Amt/unit)
1	M1	18744	Caffeine	5568747.28	0.413
2	M2	18745	Caffeine	5694384.72	0.423
3	M3	18746	Caffeine	5646098.85	0.419
4	M4	18747	Caffeine	5450694.28	0.405
5	M5	18748	Caffeine	5501403.13	0.408
6	M6	18749	Caffeine	5632460.66	0.418
Mean					0.41
% RSD					**1.67**

Tab. 1 Manual sample preparation – reproducibility and precision

Sample Set Name: MW Automated Repeatability
Sample Set Id: 17729 Result Set Id: 17808

	Sample Name	Result Id	Name	Area (µV*sec)	Potency 1 (Amt/unit)
1	A1	17812	Caffeine	5278355.59	0.377
2	A2	17813	Caffeine	5264847.66	0.376
3	A3	17814	Caffeine	5297039.30	0.378
4	A4	17815	Caffeine	5265959.19	0.376
5	A5	17816	Caffeine	5297020.61	0.378
6	A6	17817	Caffeine	5224532.00	0.373
Mean					0.38
% RSD					**0.49**

Tab. 2 Automated gravimetric sample preparation – reproducibility and precision

Unknown	Volume (mL)	Area	Wv	Wa	% of Intent
1	100	5468397	39.831	40.88	0.974339
2	100	6241589	45.6575	45.34	1.007002
3	100	4505394	32.5741	32.95	0.988592
4	100	4987986	36.2108	36.12	1.002513
5	100	6727337	49.3179	49.06	1.005257

Tab. 3 "Unknown" samples for manual linearity study

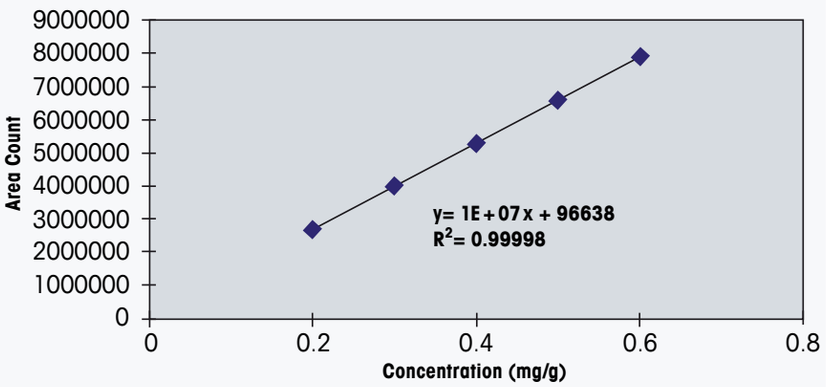

Fig. 1 Linearity plot of automated sample preparation data

Unknown	Solution (g)	Area	Wg	Wa	% of Intent
1	9.6499	3981080	2.90039	2.895	1.003751
2	9.7724	4636669	3.43293	3.42	1.005572
3	9.6779	5297030	3.89423	3.871	1.007719
4	9.6714	5926070	4.36235	4.352	1.004034
5	9.5548	6557919	4.77689	4.777	1.001583

Tab. 4 "Unknown" samples for automated gravimetric linearity study

Reproducibility and precision

The first experiment is designed to investigate the reproducibility and precision of manual sample preparation compared to the new automated gravimetric method.

Six replicate solutions were prepared by weighing 20 mg of caffeine and making it up to 50 ml in a volumetric flask using a 30:70 methanol/water mixture. This procedure took a total of 50 minutes. Dissolving/mixing the samples on a shaker took 15 minutes of this time, so the manual labour time is 35 minutes full-time equivalent (FTE) – 5 minutes per sample for each weighing and dilution plus 5 minutes for the diluent prep. Table 1 shows the data. The key metric is that the relative standard deviation of these samples is 1.67 % (Tab.1).

Table 2 shows the equivalent data from the automated gravimetric method. Six replicate solutions were prepared by weighing 5mg of caffeine and adding 12.5 g of the same diluent (30:70 methanol/water) directly into a vial. This procedure took a total of 30 minutes, consisting of 10 minutes preparation time (such as filling and installing powder and liquid dosing heads and setting up the sequence) with an additional 20 minutes running time. The RSD of these samples is 0.49 % (Tab. 2).

	Manual Prep	Automated Prep
Amount of substance	20 mg solid + 50 mL diluent	5 mg solid + 12.5 g diluent
Time	50 mins (total) 35 mins (FTE)	30 mins (total) 10 mins (FTE)
Precision	%RSD = 1.67	%RSD = 0.49

This experiment highlights three advantages of automated gravimetric sample preparation. 75 % less substance and solvent are used to prepare the sample solutions; more than 70 % of labour time is saved in the sample preparation steps and most importantly the precision is improved by a factor of more than three.

Linearity testing study

The second experiment is a linearity study. To generate the manual data, five different concentrations from 0.2 to 0.6 mg/ml were prepared in 100ml volumetric flasks, which took 60 minutes. Linear regression analysis resulted in a correlation coefficient of 0.99473.

Five unknown samples were prepared with concentrations that varied between 0.3 to 0.5 mg/mL. Table 3 indicates the actual amount of caffeine weighed into the solution (Wv) versus the amount determined from comparison with the linearity plot (Wa). The agreement between these 2 values varies between 97 and 100% (final column) (Tab. 3).

An equivalent experiment was carried out to generate the automated gravimetric data. This time the solutions were prepared in 10g solvent rather than 100ml solvent. It took 40 minutes to prepare the solutions (plus 5 mins to set up the sequence). In this experiment, the linear regression analysis resulted in a near-perfect correlation coefficient of 0.99998 (see Figure 1).

As in the manual experiment, five unknowns were prepared with concentrations that varied between 0.3 to 0.5 mg/g with the mass of each solution recorded. (Tab. 4). The final column in the "unknowns" Table 4 indicates that the actual versus determined caffeine weights for each unknown are in 100% agreement in each case.

	Manual Prep	Automated Prep
Sample size	100 mL diluent	10 g diluent
Time	60 mins (total)	45 mins (total)
Correlation coefficient	0.99473	0.99998
Unknowns (% intent)	97 – 100 %	100%

The data generated during the linearity study reinforces the superior quality of the automated gravimetric approach: 90% less substance and solvent are used; 25% of time is saved; the correlation coefficient is improved and the unknown samples are accurately identified.

Practical advantages of automated gravimetric sample preparation

The accuracy of the samples prepared is significantly improved, which will have a knock-on effect in the quality of the analytical results generated. The reduced labour time and amount of substance and solvent saved has the potential to have a dramatic impact on laboratory efficiency and running costs.

Joanne Ratcliff is Communications Project Manager for Laboratory Weighing at Mettler Toledo. With a PhD in Analytical Chemistry and 14 years at Mettler Toledo, she has extensive experience in applying automated weighing, powder dispensing and sample preparation solutions to laboratory applications in the pharmaceutical industry. Applications include capsule filling, bottling of chemicals, analytical sample preparation for QA/QC and compound management.

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"Sample preparation is an analytical workflow focus area. We are delighted to have worked with Mettler-Toledo in the development and preliminary evaluation of Quantos QX1. A targeted automated (gravimetric) sample preparation approach with the QX1 has demonstrated improved precision, reduced sample and solvent consumption and less analyst time as compared to manual approaches. We continue to work with the system to understand how and where it can be routinely used."

Gang Xue, Associate Research Fellow, Pfizer ARD