# Cleaning and Recycling Mobile Phase for Chromatographic Separations

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ABSTRACT: This work describes an environmentally friendly method to reuse the mobile phase eluent for isocratic high-performance liquid chromatographic (HPLC) systems through the use of a charcoal-packed column. In traditional systems, used mobile phase eluent is typically discarded as waste. In an effort to decrease the amount of waste generated during HPLC analyses, a recycling system was developed where separation of analytes on a traditional C18 column was followed by a mobile phase cleanup step involving a charcoal-packed column. Three different isocratic chromatographic separations were examined (each involving different mobile phase and analyte compositions). The results demonstrate that the large amount of waste produced during conventional HPLC analysis was reduced by approximately 80% when a charcoal-packed column was employed. Furthermore, reproducibility in separation performance and quantitation was not significantly affected when the recycled mobile phase was used as opposed to the fresh mobile phase.

KEYWORDS: HPLC, Waste reduction, Eluent recycling, Charcoal column, Mobile phase recirculation
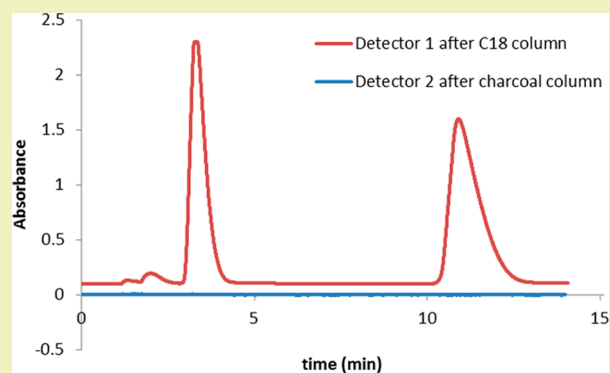
■ INTRODUCTION

High-performance liquid chromatography (HPLC) has become a mainstay technique in the chemical industry, medical testing, forensic science, academic institutions, and manufacturing.[1] As of 2006, there were roughly 180,000 HPLC units in operation around the world.[2] Assuming each HPLC unit experienced a 20 h/week operation and assuming a typical flow rate ranging 1 mL/min, nearly 11 million liters of mixed aqueous/organic waste would be produced per year. This waste often contains acetonitrile, methanol, or isopropanol mixed with low concentrations of analytes, acids, or bases and is harmful to the environment and/or toxic to humans. Disposal often requires incineration,[3] which is energy intensive and contributes to greenhouse gas emissions. The first principle of Green Chemistry[4] is that it is better to prevent the generation of waste than to treat or cleanup waste after it has been created. Several methods exist that can be used to minimize the amount of waste produced during an HPLC analysis.[5]

Solvent recycling (recirculation or reuse) is not officially condoned, but it can be employed to reduce the amount of mobile phase used during experimentation. In this practice, effluent from the column (which contains the analytes) is reintroduced into the primary mobile phase reservoir and used for subsequent analyses. This typically results in a rise in baseline over time and can affect the quality of the separation. Lee and co-workers[6] studied the effects of recirculating eluent during carbohydrate analysis and recommended that small injection volumes and large mobile phase volume help to minimize changes in retention time and peak area/height

determination. Abreu and Lawrence[7] examined the effect of the reused mobile phase on quantitation and found that unacceptably large errors existed in quantitation of individual samples, that the limits of detection were constantly changing, and that analytes with a concentration that approached the concentration in the mobile phase were often undetectable.

Automated switching can be employed as a way to remove analytes from the mobile phase effluent after separation has occurred.[8,9] Electronic systems detect a change in absorbance due to elution of some analyte that triggers a valve to switch and sends the analyte-containing mobile phase to waste. After baseline is restored, the valve switches back and the clean used mobile phase is sent to the primary mobile phase reservoir. In this way, mobile phase consumption is reduced significantly, and the problems associated with analytes in the bulk mobile phase are avoided. In addition, microscale HPLC (or uPLC) can be employed.[5,10] This technique scales down the size of the column, which results in a corresponding reduction in flow rate from 1 mL/min to about 4 μL/min. The mobile phase is not recycled. However, due to low flow rates, very little mobile phase is consumed, and little waste is produced. Unfortunately, both of these options for reduction in mobile phase use require specialized equipment and can be cost prohibitive in some applications.

One low-cost method would be to reduce column diameter and stationary phase particle size. As described in John Dolan's *Solvent Conservation*, changing the diameter size from 4.6 to 2.1 mm can decrease solvent consumption 5-fold, and changing the particle size can also result in a small decrease in solvent consumption due to a decrease in column length while maintaining the resolution of the separation.[11]

Herein, we describe the use of a charcoal-packed column to remove analyte from the mobile phase after detection so that the cleaned mobile phase can be recirculated and used in a continuous loop process. The charcoal-packed column is prepared using activated charcoal and standard steel HPLC-style tubing and fittings. Analytes in the mobile phase eluent are effectively removed by the charcoal-packed column so that the mobile phase can be reused without affecting chromatographic performance. Because of the low cost and ease of implementation of a charcoal-packed column, HPLC users can reduce costs and minimize impact on the environment without investing in new or specialized equipment. By employing this charcoal-packed column as described in this experiment, mobile phase usage was reduced by approximately 80%.

■ EXPERIMENTAL SECTION

Reagents. Acetic acid (Fisher Scientific, glacial), acetaminophen (4-acetamidophenol, Aldrich, 98%), caffeine (Kodak, >98%), DARCO activated charcoal (Sigma-Aldrich, DARCO G 60, 100 mesh), gallic acid (Sigma, >98%), *p*-hydroxybenzoic acid (Sigma, >98%), sodium hydroxide (Fisher Scientific), sodium phosphate (dibasic, Mallinckrodt, >98%), triethylamine (Aldrich, 99.5%), and vitamin B_6 (pyridoxine hydrochloride, Sigma-Aldrich, >98%) were used as received. Acetonitrile (Chromasolv, >99.9%) and methanol (Chromasolv, >99.9%) were used as received without further purification. All solutions were prepared using deionized water.

Charcoal Column Construction. DARCO G 60 steam activated carbon has a very high adsorptive capacity and finds use in pharmaceutical purification. For this reason, DARCO G 60 was chosen as the adsorptive media for the charcoal-packed column. To create a charcoal-packed column, 0.32 ± 0.02 g of dry activated charcoal was fed into a 50 mm × 4.6 mm stainless steel tube and compressed by tapping with a wooden dowel. Once packed, standard HPLC frits and fittings were used to seal the column. Loading experiments showed that this mass of charcoal could trap at least 5 mg of analyte without altering chromatographic separation. Even so, at the conclusion of an experiment, when the mobile phase and analyte system changed or when 1 mg of analyte was trapped on the column, the spent charcoal was removed from the column to waste, and fresh charcoal was repacked into the cleaned steel tube. Because of the large particle size (100 mesh, 149 μm), the backpressure caused by the charcoal-packed column was approximately 100 psi.

HPLC Parameters. Separation of all analytes occurred using a Grace Econosphere C18 3 μm column (50 mm × 4.6 mm) and was based on previously published procedures.[12−14] Mobile phase and analyte selection were based on systems commonly used in the undergraduate teaching laboratory. While the selection of these systems may not represent all possible separations, they do provide a range of solvents, pH, and buffers that demonstrate the ability of the charcoal-packed column to remove analytes and allow recycling of the mobile phase. A typical procedure to collect data related to separation performance as a function of fresh or recirculated mobile phase is shown in Table 1. All injection volumes were 20 μL, and all flow rates were 0.5 mL/min. Recirculating the mobile phase saved approximately 80% versus conventional mobile phase use.

Mobile Phase Preparation. An isocratic mobile phase was employed for all chromatographic separations used in this study. After preparation, all mobile phase mixtures were vacuum filtered through a 0.45 μm membrane and vacuum degassed for 60 min. All analyte

Table 1. Experimental Procedure Followed To Collect Data Related to Separation Performance

activity	volume of solvent used	time of analysis
preanalysis flush with appropriate fresh organic solvent	5 mL	10 min
preanalysis equilibration of the column with fresh mobile phase	20 mL	40 min
analysis of a 1 ppm standard interspersed between injections of a 250 ppm standard (to simulate heavy system use)	170[a] or 14 mL[b]	340 min
	195 or 39 mL total	390 min total

[a]A 170 mL volume was used when fresh mobile phase was employed for the analysis. [b]The same 14 mL volume of mobile phase was recirculated 12 times during the analysis phase of the experiment. The 14 mL volume included 10 mL in the mobile phase reservoir plus 4 mL in the system tubing and column.

solutions and serial dilutions were prepared with the mobile phase as the solvent. Fresh mobile phase references a system where only virgin mobile phase was employed for separations; this corresponds to a traditional approach. Recycled mobile phase refers to the mobile phase that is directed back into the solvent reservoir for recirculation after the analytes from a prior analysis have been removed by a charcoal-packed column. Reused mobile phase describes a scenario where mobile phase eluent containing analyte was simply directed back into the solvent reservoir for recirculation without any purification.

The mobile phase used to separate acetaminophen and caffeine was prepared by mixing 110 mL of acetonitrile, 4 mL of triethylamine, and 4 mL of acetic acid, and diluting to 2 L. This produced a mobile phase with a density of 0.998 g/mL and pH of 4.63. After 340 min of recirculation through a charcoal-packed column, the density of the mobile phase was 0.999 g/mL and pH was 4.61. To separate vitamin B6 and caffeine, a mobile phase was prepared by mixing 80% phosphate buffer (32 mM, pH 3) with 20% methanol. The density and pH prior to use were 0.98 g/mL and 3.3, respectively. After 340 min of recirculation, the pH was 3.5 and density was 0.99 g/mL. The mobile phase used to separate gallic acid from *p*-hydroxybenzoic acid was prepared by mixing 980 mL of 5% acetic acid with 20 mL of acetonitrile. Before use, the pH was 2.4 and ensity was 1.02 g/mL. After 340 min of recirculation, the pH was 2.5 and density was 1.02 g/mL.

Instrumentation. A single pump system, shown in Figure 1, was used to collect all data. In this system, the mobile phase was pumped from a primary mobile phase reservoir through the system using an Alltech model 712 quaternary gradient pump. Detection was accomplished using an HP series 1050 UV detector (254 nm for acetaminophen/caffeine, 292 nm for vitamin B6/caffeine, and 280 nm for gallic acid/*p*-hydroxybenzoic acid), and data were collected and processed using PC/Chrom+ software (H&A Scientific). For experimental conditions employing mobile phase cleanup and recirculation, a charcoal-packed column was installed following the detector (green path). Alternately, the mobile phase could be directed into waste (blue path, traditional HPLC) or could be recirculated without cleanup (red path). When fresh solvent was employed, the mobile phase reservoir was filled with 500 mL of the mobile phase. When recirculation was performed with either recycled or reused mobile phase, the mobile phase reservoir contained only 10 mL of the mobile phase, which was continually stirred to homogenize the solvent. A total of 14 mL of the mobile phase in the system (reservoir + tubing) was recirculated on average 12 times during the experiment as outlined in Table 1.

■ RESULTS AND DISCUSSION

Reduction in the volume of the mobile phase needed to perform a series of replicate injections for the separation of an
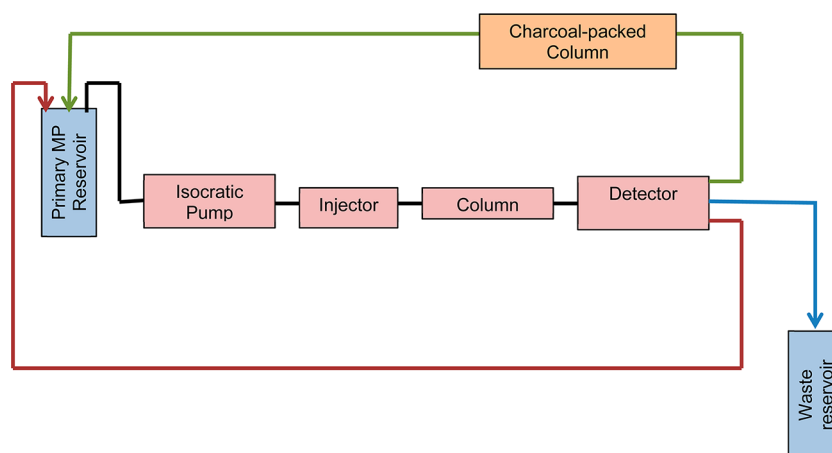
Figure 1. Single-pump HPLC system showing analysis using fresh mobile phase (blue path), mobile phase recycled through a charcoal-packed column (green path), and mobile phase reused without cleanup (red path). The black line is common to all paths.
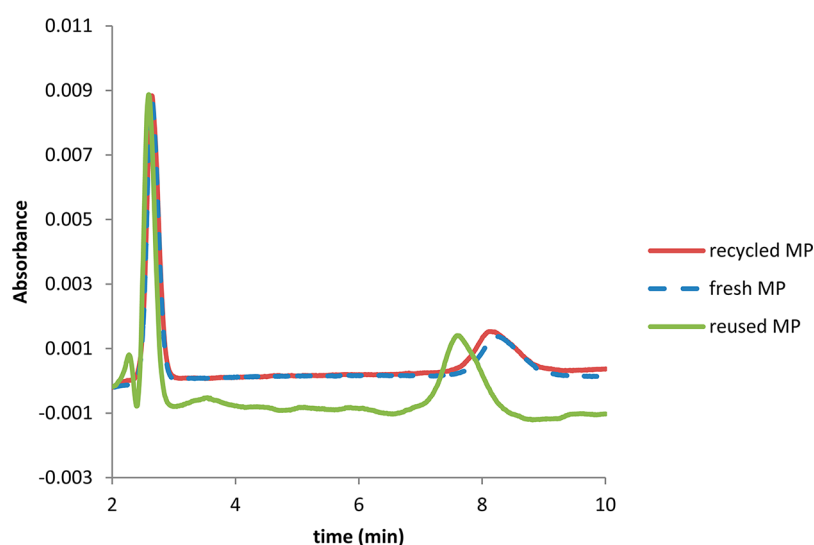

Figure 2. Comparison of chromatograms for the separation of acetaminophen and caffeine using fresh mobile phase (blue), 14 mL of mobile phase recycled 10 times through the charcoal-packed column with 90 μg of analyte loaded onto the charcoal-packed column (red), and 14 mL of mobile phase reused 10 times with 90 μg of analyte introduced into the mobile phase (green). The green chromatogram was baseline corrected for comparison purposes; the actual baseline signal was 0.177 AU. Conditions: 6:94 ACN:14 mM triethylammonium acetate buffer pH 4.6, UV detection at 254 nm, 0.50 mL/min, 25 °C, 4.6 mm × 50 mm Econosphere C18 column, dp = 3 μm, injection 20 μL, [analyte] = 1 ppm, 4.6 mm × 50 mm cleanup column packed with DARCO G 60 activated carbon, and dp = 149 μm.

analyte mixture was accomplished through the use of a charcoal-packed column that removed analyte molecules from the mobile phase after separation by the C18 column and prior to recirculation. Shown in Figure 2 are chromatograms obtained after 340 min of HPLC operation and analysis for the separation of a 1 ppm mixture of acetaminophen and caffeine using the fresh mobile phase, recycled mobile phase, and reused mobile phase. During the course of the entire experiment, the volume of the fresh mobile phase consumed was 170 mL, whereas both the recycled and reused chromatograms required only 14 mL of the mobile phase, which was recirculated 12 times during the course of the experiment. In all cases, acquisition of the chromatograms was performed after 90 μg of analyte was previously analyzed in the system. The chromatogram obtained using the fresh mobile phase was nearly identical to that obtained using the mobile phase that was recycled through the charcoal-packed column in terms of relative retention and peak quantitation (Figure 1, red and blue

lines). This demonstrates that after separation and detection, the analyte was removed by the charcoal-packed column so that the recirculated mobile phase was similar to the fresh mobile phase for every subsequent analysis. The chromatogram obtained using the mobile phase that was reused without recycling through the charcoal-packed column contained high concentrations of analyte (approximately 6.5 ppm). Although the acetaminophen peak is comparable in terms of retention time, a large break in the peak is observed due to analyte from previous injections that are present in the mobile phase. The shift of the caffeine peak and increase in area is due to buildup of analyte and contamination of the column. The presence of this analyte caused an extreme rise in the baseline signal and interfered with the separation efficiency and the ability to quantitate the peaks, resulting in poor reproducibility (Figure 1, green line).

To examine the ability of a charcoal-packed column to remove analytes from the mobile phase eluent after separation,

three chemical systems were studied. Acetaminophen and caffeine were separated on a C18 column using a weakly acidic buffer containing a small percent of acetonitrile. In a second system, vitamin B6 and caffeine were separated using a methanol-containing mobile phase. Finally, a mixture of phenolic acids was separated using a mobile phase with a high concentration (5%) of aqueous acetic acid. These systems were selected due to their similarity to many experiments found in advanced analytical chemistry laboratories at the under-graduate level.[12−15] In addition, these separation systems demonstrate the broader applicability of using a charcoal-packed column for recycling the mobile phase with different chemical compositions and pHs. Baseline stability, limits of detection, relative retention, and percent recovery studies were performed on all three systems to examine reproducibility in separation efficiency and quantitation and to demonstrate that data collected with the mobile phase that has been recycled through a charcoal-packed column and recirculated is similar to data collected using fresh mobile phase.

Baseline Stability and Limit of Detection. Shown in Figure 3 are baseline stability plots for the analysis of acetaminophen and caffeine (A), vitamin B6 and caffeine (B), and gallic acid and *p*-hydroxybenzoic acid (C). In all three systems, fresh (blue line) and recycled (red line) mobile phases show no significant changes in baseline over time or over increasing analyte concentrations in the system. This suggests that the composition of the mobile phase used for separation of each analyte pair contained no residual analyte from previous analyses. The charcoal-packed column effectively removed the analyte from the mobile phase after separation and detection, which results in a recycled mobile phase similar to the fresh mobile phase that is suitable for recirculation. However, the reused (green line) mobile phase displayed a significant increase in the baseline signal due to a large increase in analyte concentration over time.

As the baseline signal increased, higher limits of detection (LOD) were observed. LODs were determined by replicate analysis of a low-concentration standard, calculation of the standard deviation for the measured concentration, and multiplication of the standard deviation by a statistical factor (3.3) as described by Boqué and Vander Heyden.[16] Table 2 lists limits of detection for each analyte pair studied. Whether the fresh mobile phase or recycled mobile phase was used in the study, the LODs for all analytes were similar. The LODs determined for the analytes separated with reused mobile phase were much higher due to the presence of analyte in the mobile phase from prior analyses. In the case of the vitamin B6/caffeine system and the gallic acid/*p*-hydroxybenzoic acid system, the increased baseline signal prevented the detection of a 1 ppm standard. LODs were estimated by analysis of standards with increasing concentration until a reproducible signal could be observed.

Reproducibility in Separation and Quantitation. Shown in Figure 4 are plots of relative retention for the analysis of acetaminophen and caffeine (A), vitamin B6 and caffeine (B), and gallic acid and *p*-hydroxybenzoic acid (C). Relative retention factors were calculated as shown

$$\alpha = \frac{(t_{r_2} - t_m)}{(t_{r_1} - t_m)}$$

Over the course of the experiment, and as the amount of analyte introduced into the system increased, the relative
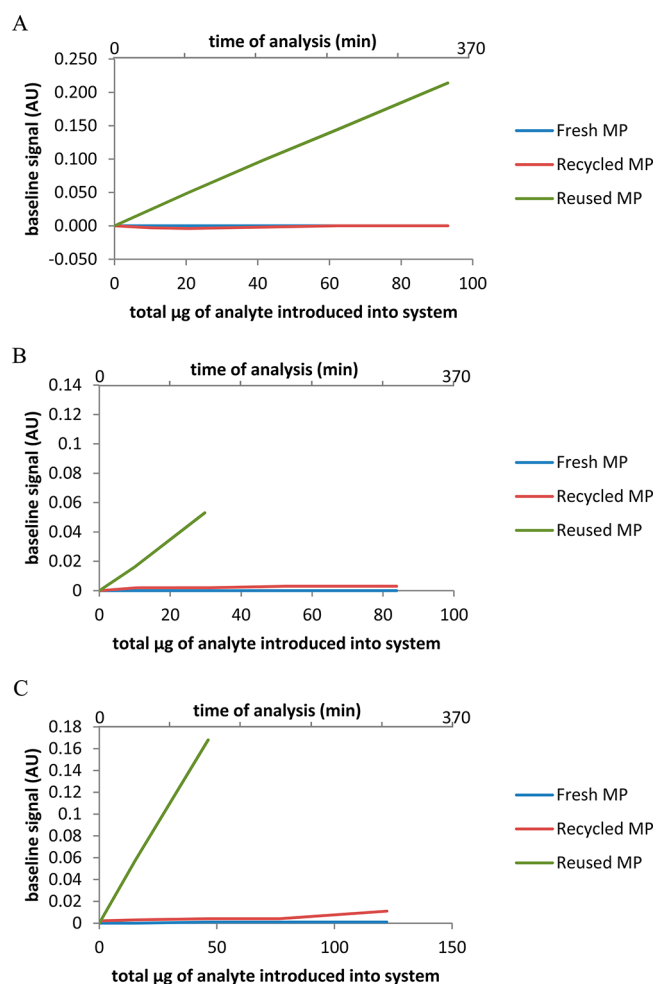

Figure 3. Influence of mobile phase recycling strategy on the detector signal for three different separations. (A) Acetaminophen and caffeine: 6:94 ACN:14 mM triethylammonium acetate buffer pH 4.6, UV detection at 254 nm. (B) Vitamin B6 and caffeine: 20:80 methanol:32 mM phosphate buffer pH 3, UV detection at 292 nm. (C) Gallic acid and *p*-hydroxybenzoic acid: 2:98 ACN:5% aqueous acetic acid pH 2.4, UV detection at 280 nm. Conditions: 0.50 mL/min, 25 °C, 4.6 mm × 50 mm Econosphere C18 column, dp = 3 μm, injection 20 μL, [analyte] = 1 ppm, 4.6 mm × 50 mm cleanup column packed with DARCO G 60 activated carbon, and dp = 149 μm.

retention for separation of the analytes in each system remained constant when the fresh mobile phase (blue line) was used. The recycled mobile phase (red line) produced a separation with a relative retention identical to that made with the fresh mobile phase, and the relative retention changed very little over the course of the experiment. This indicates that a charcoal-packed column does not significantly alter the chemical composition of the mobile phase. When the reused mobile phase was employed in the separation, the relative retention decreased, indicating that the presence of analytes in the mobile phase decrease the efficiency of the separation.

Reproducibility in the quantitation of a 1 ppm standard of acetaminophen and caffeine (A), vitamin B6 and caffeine (B), and gallic acid and *p*-hydroxybenzoic acid (C) is depicted in Figure 5 and reported as a percent recovery as a function of analysis time and as a function of the total micrograms of analyte introduced into the system. When the fresh mobile phase or recycled mobile phase was used, the percent recovery was between 90% and 110%, and the determined concen-

Table 2. Limits of Detection for Analytes Separated in Three Systems Studied[a]

	limit of detection[b] (ppm)					
	acetaminophen	caffeine	vitamin B6	caffeine	gallic acid	p-hydroxybenzoic acid
fresh MP	0.020	0.046	0.035	0.055	0.018	0.056
recycled MP[c]	0.017	0.055	0.019	0.093	0.048	0.118
reused MP[d]	0.029	0.140	2.40 ± 0.02	1.66 ± 0.08	1.4 ± 0.1	1.3 ± 0.3

[a]Small differences are noticed between the LODs determined using the fresh mobile phase and mobile phase that has been recycled through a charcoal-packed column. However, significant differences are observed when the mobile phase is reused without prior removal of the analyte. [b]LOD determined by finding the standard deviation of the concentration determined for replicate analyses of a 1 ppm standard. The standard deviation was multiplied by 3.3 to provide the limit of detection.[16] [c]LOD determined after 340 min using a charcoal-packed column to trap approximately 90 μg of analyte. The same mobile phase volume was recirculated 12 times during this period. [d]LOD for vitamin B6, caffeine, gallic acid, and p-hydroxybenzoic acid could not be determined through the analysis of a 1 ppm standard because the signal from the 1 ppm standard could not be detected above the baseline. Rather, analysis of increasingly higher concentrations was made, and an average concentration that could be detected above the baseline was determined.[16]
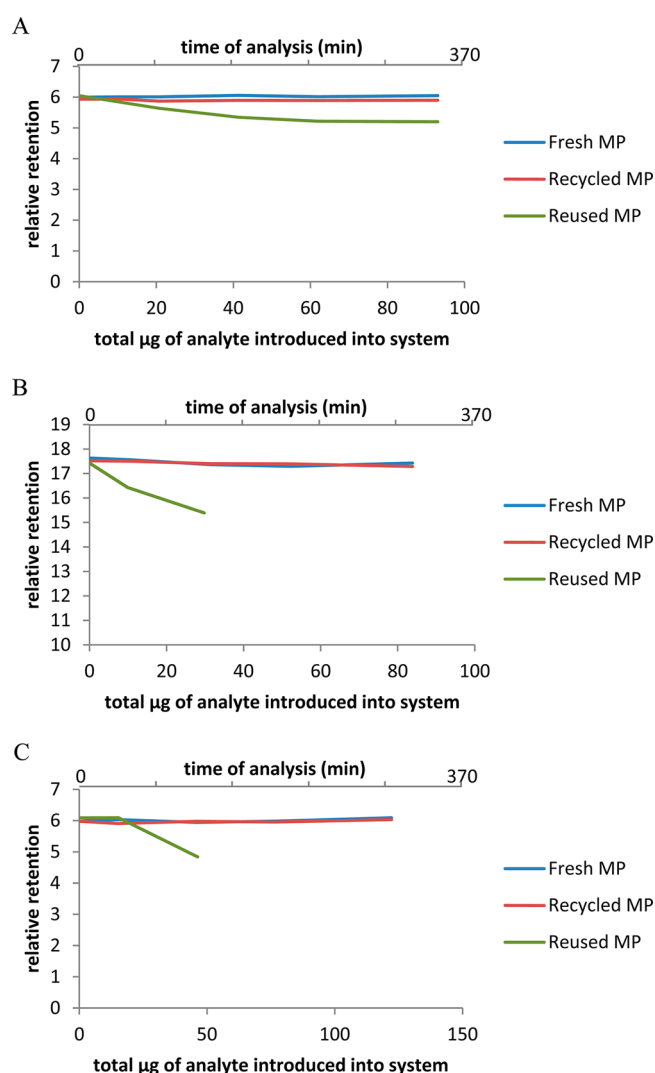

Figure 4. Influence of mobile phase recycling strategy on relative retention (see text) for three different separations. (A) Acetaminophen/caffeine. (B) Vitamin B6/caffeine. (C) Gallic acid/p-hydroxybenzoic acid. Conditions: see Figure 3.

Figure 5. Influence of mobile phase recycling strategy on percent recovery for three different separations. (A) Acetaminophen and caffeine. (B) Vitamin B6 and caffeine. (C) Gallic acid and p-hydroxybenzoic acid. Conditions: see Figure 3.

trations over the course of the experiment were consistent with a relative standard deviation that ranged from 0.4% to 4%, depending on the analyte. The ability to determine the concentration of an analyte is not significantly altered when the recycled mobile phase is used in place of the fresh mobile phase. When used the mobile phase was reused without
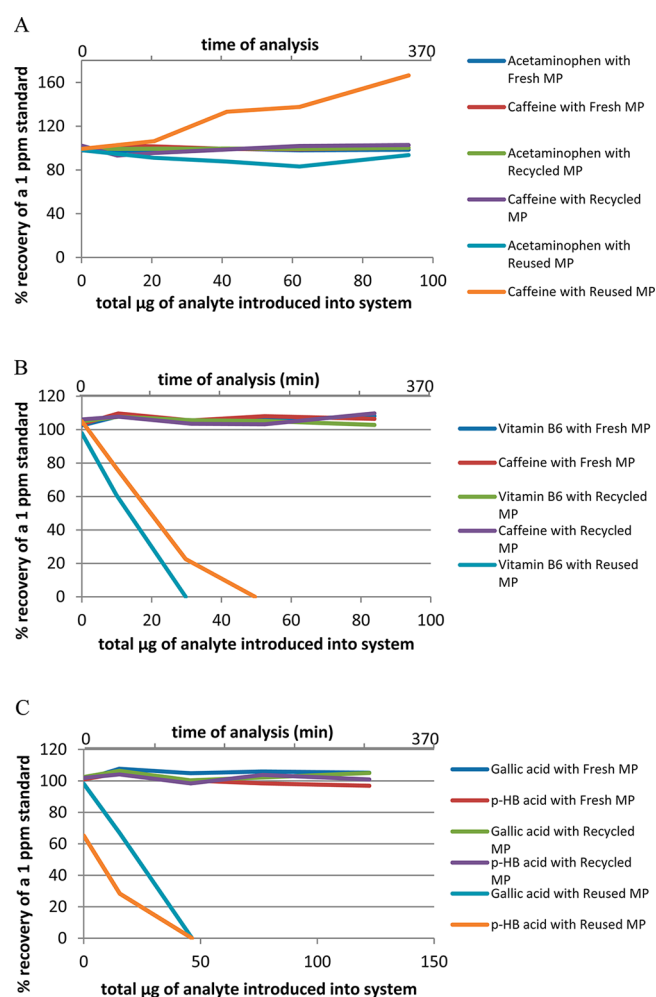
purification, the percent recoveries changed significantly, decreasing for most analytes until the 1 ppm standard could no longer be detected.

■ CONCLUSION

On the basis of baseline stability, limit of detection, relative retention, and quantitative recoveries, it is clear that the used mobile phase recycled through a charcoal-packed column can

be recirculated and reused for analytical separations, and that no significant deviations from a more traditional approach are observed. The large volume of mixed aqueous/organic waste generated using traditional HPLC is replaced with a small mass of spent charcoal waste when a charcoal-packed column is employed, with the total mass of solid waste generated dependent on the amount of analyte trapped by the charcoal. Alternately, a small volume of a high eluent strength solvent can be used to wash and regenerate the activated charcoal, although regeneration has not been explored in the current study. A charcoal-packed column has been shown to work in systems that involve different analytes and different mobile phase compositions. However, not every chemical separation has been tested, and before a charcoal-packed column is used, it should be tested on the basis of multiple figures of merit to ensure that high quality analytical data is generated.

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Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Gerbera, F.; Krummen, M.; Potgeter, H.; Roth, A.; Siffrin, C.; Spoendlin, C. Practical aspects of fast reversed-phase high-performance liquid chromatography using 3 μm particle packed columns and monolithic columns in pharmaceutical development and production working under current good manufacturing practice. *J. Chromatogr., A* **2004**, *1036* (2), 127−133.

(2) *High Performance Liquid Chromatography: Competing Techniques Setting the Bar Higher Market Forecast: 2012−2016*; Strategic Directions International, Inc.: Los Angeles, CA, August 2012.

(3) Oppelt, E. T. Incineration of hazardous waste. *J. Air Pollut. Control Assoc.* **1987**, *37* (5), 558−586.

(4) Anastas, P. T.; Warner, J. C. *Green Chemistry: Theory and Practice*; Oxford University Press: New York, 1998.

(5) Sandra, P.; Vanhoenacker, G.; David, F.; Sandra, K.; Pereira, A. Green chromatography (Part 1): Introduction and liquid chromatography. *LCGC Europe* **2010**, *23* (5), 242−259.

(6) Lee, R. E., Jr.; Friday, D.; Rojas, R.; James, H.; Baust, J. G. An evaluation of eluent recycling and column life for HPLC analysis of carbohydrates. *J. Liq. Chromatogr.* **1983**, *6* (6), 1139−1151.

(7) Abreu, O.; Lawrence, G. D. Analytical reliability of mobile-phase recycling in liquid chromatography. *Anal. Chem.* **2000**, *72*, 1749−1753.

(8) Alltech Associates, Inc.. *Anal. Chem.* **1992**, *64* (21), 1025.

(9) Phenomenex.. New model HPLC solvent recycler from Phenomenex. *Chromatographia* **1996**, *43* (11/12), 684.

(10) Swartz, M. E. UPLC: An introduction and review. *J. Liq. Chromatogr. Relat. Technol.* **2005**, *28*, 1253−1263.

(11) Dolan, J. Solvent conservation. *LCGC North America* **2007**, *25* (3), 268−274.

(12) Leacock, R. R.; Stankus, J. J.; David, J. M. Simultaneous determination of caffeine and vitamin B$_6$ in energy drinks by high-performance liquid chromatography. *J. Chem. Educ.* **2011**, *88* (2), 232−234.

(13) Ferguson, G. K. Quantitative HPLC analysis of an analgesic/caffeine formulation: Determination of caffeine. *J. Chem. Educ.* **1998**, *75* (4), 467−469.

(14) Ghiselli, A.; Nardini, M.; Baldi, A.; Scaccini, C. Antioxidant activity of different phenolic fractions separated from Italian red wines. *J. Agric. Food Chem.* **1998**, *46* (2), 361−367.

(15) Brenneman, C. A.; Ebeler, S. E. Chromatographic separations using solid-phase extraction cartridges: Separation of wine phenolics. *J. Chem. Educ.* **1999**, *76* (12), 1710−1711.

(16) Boqué, R.; Vander Heyden, Y. The limit of detection. *LCGC Europe* **2009**, *22* (2), 82−85.