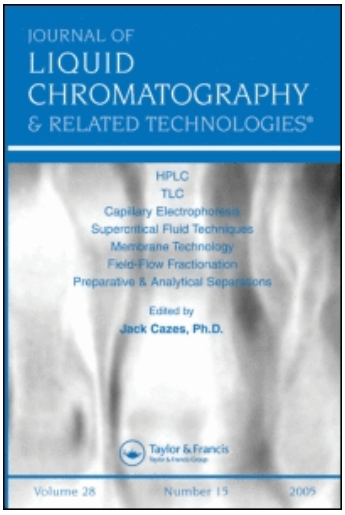

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High Performance Liquid Chromatographic Analyses of Sulphonamides and Dihydrofolate Reductase Inhibitors. IV. Recoveries from the Stationary Phase, Quantitation and Sensitivities

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSES OF SULPHONAMIDES AND DIHYDROFOLATE REDUCTASE INHIBITORS. IV. RECOVERIES FROM THE STATIONARY PHASE, QUANTITATION AND SENSITIVITIES

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ABSTRACT

Of the twenty-two sulphonamides and three commonly used dihydrofolate reductase inhibitors investigated for reverse phase separation in previous studies, the recoveries of five representative drugs from the stationary phase have been examined. Sulphanilamide, sulfisomindine, sulphaquinoxaline, diaveridine and pyrimethamine were chosen. Peak areas off the analytical column were compared with those obtained by substitution with stainless steel tubing. After careful correction for flow rate differences where necessary, 100% recoveries were indicated. As a further check, Maloprim tablets were assayed for pyrimethamine and the analysed results were found to be (99.1 ±

2.9)%. Limits of detection (LOD) for aqueous standards varied from 0.67 (for sulphanilic acid) - 0.03 μg mL^{-1} (diaveridine). The linear dynamic range extended from the LOD for each drug to >5 μg mL^{-1}.

INTRODUCTION

In the previous papers in this series, the reverse phase retention behaviour of twenty-two sulphonamides(SFA) and the three commonly used dihydrofolate reductase inhibitors(DHFR) have been examined. The objective was to see if a full separation of the 25 drugs could be achieved and HPLC could be used for broad screening purposes. Phosphate buffers were adopted throughout. In general, sulphathiazole (ST) and sulphapyridine (SP) coelute. Also, in the middle of the chromatograms, sulphameter (SM), sulphamoxole (SAM), sulphamethazine (SMAZ), diaveridine (DVD) and sulphamethizole (SMIZ) were only partly separated under the majority of conditions investigated throughout the extended study.[1-3] These five compounds were generally tightly bunched, usually included more than one coelution and often overlapped with the next compounds to elute. In methanol modified mobile phases,[1] in the optimum pH range of 2.7 and 3,[4,5,1] the best gradient separated 19 of the drugs and 2 hydrolysis products with $R_s \geq 1$[6] and 2 more drugs with $R_s \approx 0.9$. ST and SP had $R_s \approx 0.65$, and, SAM and SMAZ were not separated. Higher pHs are generally unfavourable and lead to excessively congested chromatograms. However, due to differences in $pK_{a,2}$ values, SM, SAM, SMAZ, DVD and SMIZ were baseline resolved from each other at pH 6.5. At low phosphate concentrations (0.001 M), the last 10 drugs can be eluted almost perfectly. At higher phosphate concentrations (0.01-0.1 M), the front end of the chromatogram is improved.

Acetonitrile modified mobile phases provided some promising selectivity differences, but these were countered by other losses of resolution.[2] Attempts to incorporate these beneficial differences into MEOH gradients were unsuccessful. Returning to MEOH modified mobile phases, combined flow and solvent programming resolved the first 13 compounds, including the seldom separated ST and SP ($R_s \approx 1.1$).

In the third stage,[3] the effects of a competing base (tertiary butyl ammonium phosphate) were not found to be helpful. However, with the exception of SAM and SMIZ, ion pairing (heptane sulphonic acid at 0.5 and 1.0 mM) allowed the separation of all other pairs of compounds with $R_s \geq 0.9$. The result is clearly superior to any previous HPLC separation.

In summary, the simultaneous separation of all 25 drugs was not achieved. However, most combinations of most of the drugs were separable under some conditions.

The final stage of this study is concerned with quantitation. In spite of the long recognised problems of irreversible adsorption in GSC, related phenomena in LSC (albiet to a lesser degree), tailing and band-broadening on silica and silica-based phases in all areas of chromatography and monumental efforts to produce deactivated silica surfaces and polymeric coatings to cover the silica support, it is often presumed in reverse phase HPLC (RPLC) that what is injected will be detected. In more recent times, it has been accepted that SFE recoveries of non polar substances from solid matricies (for example, polycyclic aromatic hydrocarbons from urban dust, fly ash and river sediment)[7] are greatly enhanced by the presence of co-solvents such as MeOH which compete for active surface sites. And for some of the analytes, recoveries were still poor. For polar analytes, the situation is far worse. Above 10% MeOH in supercritical CO_2, 90% recoveries of SFA from sand were readily achieved.[8] Greater than 90% recoveries were difficult and recoveries only very slowly and asymptotically approached upper limits with increasing time or severity of extraction conditions (pressure, % co-solvent). One hundred per recoveries were never obtained.

In our initial investigations of the separation of SFA and DHFR (using an alternative stationary phase)[5], losses of both classes of analytes occurred. In the case of the SFA, large (46-73%) losses occurred, but the amounts seemed to vary randomly with respect to elution order and the polarity of the SFA. The reduced amounts of the SFA eluted in a normal fashion, in comparable amounts in successive runs. As far as could be determined, the DHFR were not eluted, irrespective of the number of injections or the strength of the mobile phase. Only in the presence of EDTA did the DHFR elute, and then, at the solvent front in amounts dependent upon the concentration of EDTA. In view of the negligible effect of the EDTA upon the retention of the SFA and the retention of DHFR on alternative reverse phases, the implication apppeared to be that the mechanisms of the 'irreversible adsorption' and the reverse phase retention were different. This was interpreted to mean, that EDTA underwent equilibrium chelation with metal impurities responsible for the 'irreversible adsorption' and formed soluble complexes with the EDTA that had a high charge to mass ratio, thus preventing reverse phase retention.

In view of all of the above, the presumption of 100% elution of any of the SFA or DHFR from any silica-based stationary phase would be unwise. However, this is seldom directly checked. Alternative strategies are usually adopted.

The recovery of drugs from complex liquid media such as biological fluids (urine, serum) or tissues is a demanding task and recently published assays for the SFA and DHFR[9-20] are representative of the experimental methods practised generally. Spiking of otherwise identical, but uncontaminated matricies provides a method of estimating recoveries to validate the combined work-up, extraction and liquid chromatographic analytical procedures. Alternatively, where recoveries are not measured, the determination of unknowns relative to spiked, like matrices, removes many of the sources of potential error. All of the assays mentioned above have used spiking. Standard additions - routinely used in atomic absorption but seldom used in LC[10] - would clearly provide a higher level of protection from more subtle effects possible due to an imperfect match of matricies. The (frequent) use of internal standards (IS),[11-13,15,18,20] goes much of the way to fulfilling this role and to compensate for systematic variations in instrumental conditions. However, no IS can perfectly match the target analyte so that there will always be some element of residual risk. An example of this potential problem, is provided by SFE data for the SFA.[8] Recoveries from sand and spiked, homogenised tissues varied greatly between the five SFA investigated. Furthermore, the dependence of recoveries upon extraction variables (%MeOH, pressure, time and matrix) was not the same for each SFA. SMIZ was by far the hardest of the five SFA to extract from sand and might therefore be expected to be most likely to give rise to problems associated with adsorption on the silica support. Recoveries of sulphamethoxazole (SMOX) from sand were far more sensitive to temperature than for the rest. Both of these were difficult to recover from fortified homogenate on sand at temperatures near optimal for SMOX, thus indicating SMOX to be most subject to matrix interactions. As the presumption of 'like physico-chemical behaviour' is made of an IS, detailed knowledge of the interactions of an IS and the target analytes with the intended environments is highly desirable. But this is not generally the case. The usual guidelines of identical functionality and minimal size difference for an IS seem essential. The combination of matrix matching and internal standardisation is likely to eliminate the vast majority of sources of error, especially when combined with checks of system equilibration and reproducibility via repetition studies.

One such set of circumstances that could give rise to difficulties, is the measurement of a target analyte in quantities near the limit of detection (LOD), especially with the use of a new column. Even for small losses on the stationary phase, positive results would become false negatives. In this region of the LOD, a small shift in the LOD might not be considered of significance. Due to differing physico-chemical properties, the use of an IS would not necessarily help. In this study, we therefore directly examine recoveries from the stationary phase.

EXPERIMENTAL

Chemicals and Solutions

All chemicals and solution preparation (stock solutions 25 ng/μL of each SFA and DHFR in 4% methanol and mobile phases) were prepared as described previously.[1,3] The full names and abbreviations for all of the analytes were given in Part I of this study.[1] Structures of all of the drugs have been listed previously.[21]

Maloprim tablets, containing a specified 12.5 mg of pyrimethamine (PYR) each, were purchased from a local pharmacy. Five sample solutions were prepared from separate, single tablets. Each tablet was dissolved in methanol by vigorous shaking and sonication and then diluted to 100 mL with methanol (solution A). Various dilution procedures were tried, but the one that yielded the largest peak areas was adopted. Twenty mL of solution A was blown down to dryness with nitrogen, re-dissolved with sonication in two mL of methanol and made up to 100 mL with Milli-q water (25 ppM). Five independent standards containing approximately 25 ppM PYR were prepared by dissolution of PYR solid in methanol with sonication and dilution to the same 2% methanol Milli-q water. All PYR solutions were filtered through a Millipore 0.2 μm HA filter prior to injection.

For the determination of detection limits, the 25 ng/μL, 4% MEOH standard solutions of the drugs were then diluted further to prepare final concentrations of the SFA and DHFR at exactly 15, 9, 5, 2.5, 1, .5, .25 and .2 ng μL^{-1}. Additional methanol was added to maintain a final 4% MEOH concentration.

Instrumental Configuration

A full description of the modified Varian (Walnut Creek, CA, USA) LC with split flow and packed capillary columns has been previously described.[1] Figure 1 is a schematic of it. A is the packed capillary and B is the parallel conventional column for the diversion of the majority of the mobile phase which was pumped at 1.00 mL min^{-1}. This was the normal configuration as used for all analyses. To check recoveries from the stationary phase, A was replaced with an equal length (30 cm) of 0.13 mm id stainless steel tubing (sst) and B was modified to achieve the same flow rate (~ 6 μL min^{-1}) through the detector. This was ultimately achieved by bending a piece of the same id sst

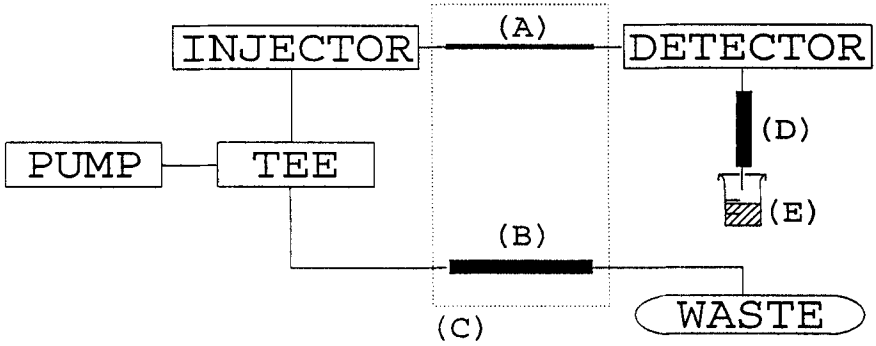

Figure 1. Instrumental configuration for the study of recoveries from the stationary phase. 1. For the analytical system: (A) 0.35 mm i.d. x 300 mm packed capillary column; (B) 4 mm i.d. x 300 mm column; (C) column oven set to 31°C; (D) 4 mm i.d. x 150 mm column; (E) measuring cylinder for flow rate determination. 2. For the alternative configuration without retention: (A) 0.13 mm i.d. x 1/16" o.d. stainless steel tubing; (B) as 2(A) except sharply bent to provide a constriction; (C), (D) and (E) as above.

and hammering it flat until the desired result was obtained. A reduced pumping rate of 0.15 mL min^{-1} was required and the resultant measured flow rate through the flow cell was 5.7 μL min^{-1}. The detector was set at 270 nm.

Chromatography

The protein C_{18} columns were 30 cm stainless steel, 0.35 mm i.d. and 0.48 mm o.d. and were packed by Varian. The stationary phase was the Separations Group Vydac IDI-TP 5 μm ± 1 μm (75%) silica with surface area 80 m^2/g, pore volume 0.63 cm^3/g and average diameter of 330 Å. The C_{18} bonded phase was TMS capped with a total carbon loading of 6-7%.

All experiments were performed at a column oven temperature of 31 Celcius, at flow rates of approximately 6 μL min^{-1} through the detector. For the recovery studies from the stationary phase and the analysis of Maloprim, isocratic elution was utilised. The mobile phase was 75% (0.001 M phosphate buffer, 0.5 mM with respect to heptane sulphonic acid (HPSA), pH 2.95) and 25% methanol. In the case of the detection limits, the best previously determined gradient (Table 5, Part III of this study)[3] was employed. It is a complex gradient, pH 2.95, 0.001M phosphate with the percentage of methanol

varying from 0-70%. The 1 mM HPSA aqueous phase was substituted by an otherwise identical aqueous phase 0.5 mM with respect to HPSA over the 8-20 minute interval. Precise flow rates were determined by timed collection of the eluent over several hours in a semi-sealed environment.

RESULTS AND DISCUSSION

Recoveries from the Stationary Phase

Of the 25 drugs examined in this study, it was desirable to pick a representative group for the recovery studies. As potential problems with the two classes of compounds might be expected to be different,[5] it was necessary to choose analytes from each class. Also, losses can occur due to a variety of mechanisms. For example, in an unfortunate choice of sample filters it was found that the 22 SFA were removed in progressively larger amounts in proportion to their elution time. For the small, more polar, early eluting drugs there appeared to be little or no loss. The intermediate eluters gave peaks about half the size to those from an unfiltered solution and the late eluters were absent from the chromatograms.[22] Hydrophobic interactions were clearly implicated, so the size range of the analytes must be represented in any recovery study. To represent this range of sizes/polarities, three SFA were chosen: sulphanilamide (SAN, early eluting), sulfisomindine (SISM, intermediate eluter) and sulphaquinoxaline (SQ, late eluter). Although only three DHFR were used in the current study, the problems previously observed with their losses have been more severe.[5] Hence, the earliest eluter in the group (diaveridine, DVD) and the late eluter (pyrimethamine, PYR) were included.

Isocratic elution was chosen not only to minimise the turnaround time between runs, but also to ensure that random fluctuations in the solvent composition and thus, minor shifts in the wavelengths of maximum absorbance and variations in the molar absorptivity were kept to absolute minima. Isocratic elution is also necessary in order to be able to make unequivocal corrections for flow rate fluctuations. For the fixed pumping rates adopted (1.00 mL min^{-1} for the normal configuration with the packed capillary and conventional columns in parallel), it was observed that the actual (measured) flow rate varied slightly. This was usually only between 5.8 and 5.9 μL min^{-1} from day to day and generally stayed constant once established each day. However, when the columns were replaced with sst the closest measured flow rate obtainable was 5.9 μL min^{-1}.

The ramifications of variations in flow rate are twofold. There is firstly the changed residence time in the detector, and secondly, an altered volume of mobile phase able to flush the automatic injector loop in the programmed interval. At the average, measured flow rate of 5.9 μL min^{-1}, one loop volume of 1.0 μL is pumped through the analytical side of the split-flow system in 0.17 minutes. Checks of the variation in peak areas with the injection interval showed that virtually complete delivery of the loop contents was achieved by an injection interval of 0.19 min. (1.1 μL). Any trace of analytes after that time would be better left in the loop. Minor variations in the amount of sample delivered from the loop would be compensated for by calibration for flow rate whereas, longer injection intervals and peak tails could decrease the reproducibility due to the high level of uncertainty associated with the integration of the extended part of peak tails.

The effect of flow rate upon peak area was determined by varying the pumping rate. To achieve this the columns were removed and replaced with the sst. As there was no retention, the peak shapes for all compounds were the same and the choice of analyte was immaterial. SAN was used. There is a simple linear relationship between the set pumping rate (spr/mL min^{-1}) and the actual flow rate (afr/μL min^{-1}) through the detector,

$$afr = -1.289 + 46.79spr$$

and the correlation coefficient is 0.9979. The variation of the SAN peak areas $(A_{SAN})/10^3$ with afr is given in Figure 2. (The differently shaded points represent different numbers of measurements.) Via SigmaPlot V4.1 the relationship was found to be:

$$A_{SAN} = (23.68afr^2 - 352.8afr + 1644)x10^3 \quad (1)$$

Table 1 shows the quintuplicate peak area measurements and the means and standard deviations for each of the five selected analytes. Column 2 contains the areas measured after elution from the packed capillary, corrected to a flow rate of 5.9 μL min^{-1} with the aid of equation 1, where necessary. Column 3 shows the raw data obtained from samples passed through the sst at 5.7 μL min^{-1} and in column 4 are the equivalent data after correction to 5.9 μL min^{-1}. Comparison of columns 2 and 4 shows that each mean falls within the range of (the other mean + its standard deviation (sd)), and generally, a long way within one sd.

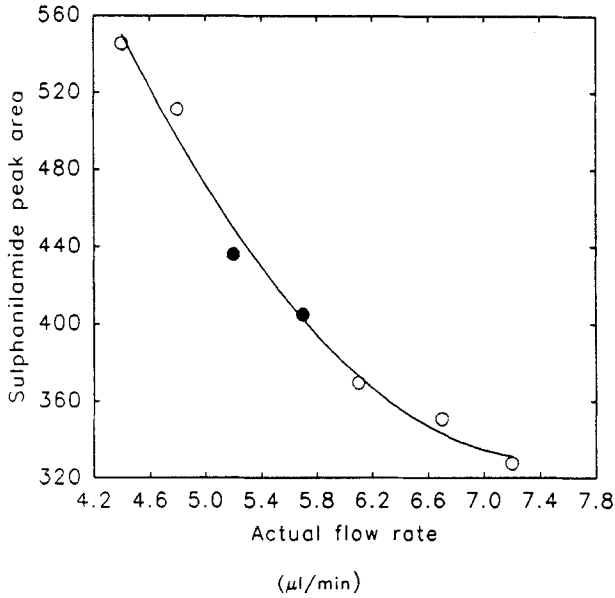

Figure 2. Variation in the detected area of sulphanilamide ($/10^3$) as a function of the flow rate.

The application of a t-test or some other statistical process such as the analysis of variance (for the two variable factors of 'column' and drug), ultimately depends upon the null hypothesis. That is, that the two sets of data are spread about the same population mean. When a test is done and the null hypothesis is rejected, difference outside of random variation is proven at some confidence level.[23] On the other hand, if the null hypothesis is accepted the converse conclusion does not follow. The sets of data are not proven to be 'the same'. For sets of data that are progressively more divergent, it may be shown that differences continue to have a higher (percentage) significance. Again, the converse is not true.

It is not possible to demonstrate a statistical difference between the data of columns 2 and 4 for the normal range of p values (or percentage confidence levels: 0.1-0.005 (or 90-99.5%). The null hypothesis is proven in all cases.

To demonstrate the 'sameness' of two sets of data is not simple. In the end, random variations dictate that sets of data will not be the same and degrees of sameness are not addressed. Statistics is largely concerned with the

Table 1

Peak Area Measurements after Retention on the Analytical Column and Without Retention*

	Peak Areas		
		Stainless Steel Tubing	
Compound	**Capillary**	**Measured**	**Corrected**
Sulphanilamide	388362	407487	285529
	386132	417252	394768
	384420	405707	383845
	389545	407206	385264
	381544	406776	384857
	386001 ± 3182[a]	**408886 ± 4726**	**386853 ± 4471**
Sulphisomidine	467804	497087	470301
	466405	495643	468935
	470666	500775	473791
	460979	494481	467836
	460488	486801	460570
	465268 ± 4419	**494957 ± 5138**	**468286 ± 4861**
Sulphaquinoxaline	299665	320729	303446
	304768	329106	311372
	306701	326718	309113
	316376	319389	302179
	305826	328391	310696
	306667 ± 6073	**324867 ± 4498**	**307361 ± 4256**
Diaveridine	221326	240419	227464
	230881	240913	227931
	229731	230123	217723
	221177	229056	216713
	223689	239175	226207
	225361 ± 4641	**235937 ± 5841**	**223224 ± 5527**

Table 1 (continued)

Peak Area Measurements after Retention on the Analytical Column and Without Retention*

	Peak Areas		
		Stainless Steel Tubing	
Compound	**Capillary**	**Measured**	**Corrected**
Pyrimethamine	237628	247182	233862
	228964	232063	219558
	223312	241491	228478
	227177	242366	229306
	226321	239667	226752
	228680 ± 5403	**240554 ± 5499**	**227591 ±5203**

* All areas are corrected to a standard flow rate using Equation 1.

[a] Average ± s.d.

definition of legitimate difference. In an attempt to demonstrate sameness, we have calculated confidence intervals (ci) associated with approximately the lowest confidence levels for inclusion of the population mean. For SISM, it is possible to go as low as the 90% confidence level to ensure that the mean area measurement off the column still lies within the ci off the sst, and vice versa. This means that for the distributions implied by the means and sd's for SISM, there is only a 90% confidence of this range including the (overall) population mean. However, the mean from the alternate measurement on SISM is still within the range. In the case of the other drugs, the approximately lowest ci [24] which includes the alternative mean (and vice versa) have levels of confidence of 80% for (DVD), 70% for (SAN and PYR) and 65% for (SQ). This sort of concordance between the two sets of data for each drug when there is such doubt about inclusion of the population mean, is interpreted to indicate a high degree of agreement.

Careful inspection of the data in Table 1 does reveal some systematic variation. For the three SFA, the areas of the compounds off the sst are all marginally larger (SAN, +0.2%; SISM, +0.6%; SQ, +0.2%) than those off the analytical column. The reverse is true for the DHFR (DVD, -0.9%; PYR, -0.5%). However, these differences are so far within the sd's that it must be

concluded that there were not detectable losses of any of the drugs on the stationary phase. There appears not to be any significant dependence upon drug type or elution order and therefore polarity.

It should also be noted that no effort was made to ensure the similarity of peak shapes in the comparison and indeed the adoption of a constant isocratic regime over all analytes ensured that the recoveries for most drugs were based upon dissimilar peak widths. As has been found by others,[25] peak shape is immaterial provided the flow rates and mobile phase are constant, and that integration is 'complete'.

Analysis for Pyrimethamine

As a check of the recoveries, we have chosen to analyse for pyrimethamine (PYR). This drug was chosen since the DHFR appeared to be more susceptible to loss than the SFA. As residue analysis was beyond the scope of this particular study, a pharmaceutical product (Maloprim) was chosen.

The exact concentrations of the PYR standards (in mg L^{-1}), the area counts and the response factors (in mg L^{-1} per unit area count / 10^{-5}) of the five independent standards were 25.06, 323805 and 7.739 for std. 1; 25.10, 324810 and 7.728 for std. 2; 24.92, 320534 and 7.775 for std. 3; 25.96, 326382 and 7.954 for std. 4; 25.38, 322803 and 7.862 for std.5. Hence, the mean response factor ± sd (σ_{n-1}) was (7.812 ± .095) x 10^{-5}. Using this mean and the five solutions of a Maloprim tablet, the mean recovery (relative to the stated 12.5 mg per tablet) and the overall sd were (99.1 ± 2.9)%.

Within recent years there have been few determinations of the SFA and DHFR reported in pharmaceuticals. Three of these include a CE determination with β-cyclodextrin modifier,[26] one HPLC determination with amperometric detection[27] and an MECC analysis employing an internal standard.[28] The recovery rate obtained above is well within the agreement found between the analytical result and stated composition as found in these other studies. It also supports complete recovery from the stationary phase, in the case of PYR.

The precision of this recovery study requires some comment. It is obvious from the response factors quoted above, that there is a systematic trend underlying the random fluctuations in values. The RSD is 1.2%. As the afr was of the order of 6 μL min^{-1}, several hours were required to collect an appropriate volume for measurement with reasonable certainty. The problem with this was that there was only an average rate determined. Fluctuations in

the flow rate could not be ascertained. As an alternative check of the flow rate, the (slow) drip rate was timed and correlated with the volumetric flow rate. This was then used as a quick daily check of the system. However, we did not analyse the range of drip rates about the correlated mean values. Hence, it is possible that one of the significant contributers to the RSDs observed would be minor variations in the flow rate. The minimum possible decrements in the spr were 0.01 mL min^{-1}. From 0.15-0.14 mL min^{-1} at the pump, the afr changed from 5.7-5.4 μL min^{-1} and the areas recorded for SAN increased from 404993-436095; about 7.7%. Minor changes in this range could easily account for the 1.2% RSD. There are two possible sources of flow rate variation. The first is the pump. The second is derived from the experimental design because the restrictor column (D in Figure 1) was not housed in the oven, thus allowing the mobile phase to change in temperature and viscosity and thus, alter the flow rate (according to ambient conditions) before measurement.

For the solutions of the Maloprim tablets, the peak areas varied in a similar fashion to the response factors for the standards, but over a larger RSD (2.6%). Dissolution inconsistencies may also contribute. For a greater level of precision, it would be necessary to enclose the restrictor column in the oven and intersperse the standards and samples, draw a trend line from the standards and take response factors from that line of best fit. A dynamic on-line flowmeter and a study of dissolution kinetics would also enhance the technique. However, there was no point in further refinement for the current study.

Detection Limits and the Linear Dynamic Range

In order to establish approximate values for the limit of detection (LOD) for each drug and to investigate the linear dynamic range (LDR) for the micro scale LC system, the range of standards chosen was concentrated at the dilute end (0.2, 0.5, 1.0, 5.0. 15.0 and 25.0 ppm).

LOD is defined as the minimum concentration or amount of analyte that can be detected with reasonable certainty for a given analytical procedure.[29] The problems associated with the accurate definition of detection limits and its confusion with other concepts in trace analyses has been reviewed.[30] The current standard method for estimating the detection limit in an analytical procedure is when the peak height of the analyte is three times the standard deviation of the baseline noise.[29-32]

The detection limits are thus determined by baseline noise fluctuations and the width of the eluting band. A reduction in both of these factors will greatly improve the detection of trace amounts of analytes. Hence, gradient

elution which provides narrow peaks thoughout the entire run was employed. Baseline noise was determined as the height of the largest noise fluctuation in a pre-selected chart time interval.[30] The 60 minute analysis time for the IPC gradient run was divided into four chart time interval sections. In each of these sections, the height of the largest noise fluctuation was measured. Hence, the noise value used in the signal-to-noise ratio (S/N) calculation for each analyte peak was based upon which chart interval section the analyte peak emerged within and the attenuation of the chromatogram.

Using the low concentration data points for each drug, the approximate 'concentration LODs' in ppm are: SNAC, 0.67; SCP, 0.32; SP, 0.29; SDZ, 0.28; ST and SMP, 0.27; SMOX, 0.25; SMAZ, 0.22; SMRZ and SST, 0.20; SAM, 0.19; SG, SISM and SMIZ, 0.18; SM, 0.16; PST, 0.12; TMP, 0.11; SAN, SISX, SDIM and PYR, 0.10; SAC and SB 0.08; SQ, 0.07; DVD, 0.03.

For a comparison of these LOD for the micro LC system with values for a conventional LC system, it is necessary to find data obtained by UV absorption at similar wavelengths. Only two of the recent studies fit this prescription. Using 4.6 mm id columns, LODs obtained for aqueous standards in ppm are: SMOX, 0.035 (271 nm, 20 μL injected),[19] and, SG, 0.08; ST, 0.07; SP, 0.05; SMOX, 0.04; SDZ, 0.03 (260 nm, 50 μL injected).[10] In the case of SMOX, the agreement between these two studies is notable. Compared to the micro system, the sensitivity of the conventional system is approximately 2, 4, 6, 6 and 9 times that of the micro system, for the compounds in the order listed. Some of this variation from compound to compound is due to the different monitoring wavelengths. However, the difference is clearly about half an order of magnitude. This is a clear demonstration of the higher concentration sensitivity of conventional systems which arises from the compatibility with larger sample sizes. As the micro injector used in this study delivered only 1 μL of sample, there were 20- and 50-fold advantages in sensitivity in the studies using the conventional systems, respectively. Since gradient elution was used in each of these studies, an analysis of peak widths would be necessary to determine the effect of this factor in the relative sensitivities of the micro and conventional systems. The restricted radial diffusion in the micro system would play an ameliorating role with respect to the sample size advantage of the conventional system. However, with vastly different sample sizes, much of the advantage of the greater mass sensitivity of the micro column is lost. Plots of peak height or S/N ratio versus concentration clearly show curvature below 15 ppm of the analytes but are linear to beyond 5 ppm. Insufficient data points were used to be more definite. Thus, the LDR is limited and extends only from the 'concentration LODs' for each compound to about 5 ppm; ranges of only 1-2 orders of magnitude, generally between 25 and 50.

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