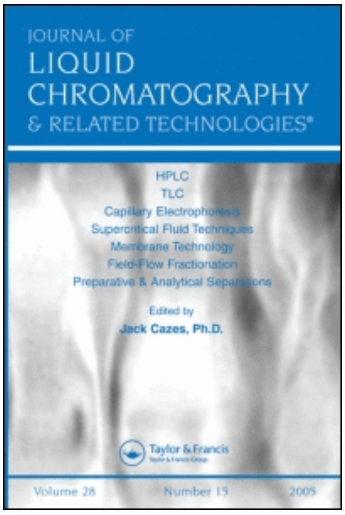

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Precision in Laboratory Robotic—HPLC Systems

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PRECISION IN LABORATORY ROBOTIC - HPLC SYSTEMS

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Abstract

Laboratory robotic systems have been used extensively to prepare samples for HPLC analysis. This review compares the precision of manual versus robotic HPLC sample preparation procedures (both simple and complex) for a variety of samples including pharmaceuticals, foods, polymers and biological fluids.

Introduction

New technology for analytical measurement and data reduction has developed at a rapid rate, but sample preparation technology has not kept pace. The great emphasis on instrumental measurement techniques often obscures the importance of sample handling and preparation in achieving quality analytical results. Today, sample preparation is the weak element in analytical methods (1,2).

Most samples for HPLC analysis require several preparation steps prior to injection. Subtle differences between analytes, interferences and

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matrices give rise to a variety of sample preparation methods, so automation must be flexible to be generally useful.

TABLE 1

Laboratory Unit Operations

LUO Class	Definition	Examples
Weighing	Quantitative measurement of sample mass	Direct measurement on balance
Grinding	Reducing sample particle size	Homogenizing
Manipulation	Physical handling of laboratory materials	Pouring Capping and uncapping containers Sample movement
Liquid Handling	All physical handling of liquids - reagents & samples.	Dispense, Dilute & Pipet Transfer (pumping & valving)
Conditioning	Modifying and controlling the sample environment	Time (start & stop) Temperature (heat & cool) Atmosphere (vacuum & gas blanket) Agitation (mix,stir,vortex & shake)
Measurement	Direct measurement of physical properties	pH, Conductivity, etc. Absorbance, Fluorescence, etc.
Separation	Coarse mechanical and precision separations	Filtration - all techniques Partition - liquid-liquid & liquid-solid Centrifugation Precipitation Distillation & Recrystalization Electrophoresis
Control	Use of calculation and logical decisions in laboratory procedures	Calculate and dispense reagent to dilute to a given concentration based on weight or volume. Adjust sample to desired pH.
Data Reduction	Conversion of raw analytical data to usable information	Peak integration Spectrum analysis Molecular weight distribution
Documentation	Creating records and files for retrieval	Notebooks Listings & Computer Files

Automating sample preparation requires automating a

sequence of laboratory unit operations (LUO's) as described in Table 1.

A particular analysis would not require all of these operations but generally 3-5 unit operations. A second analysis could not only require different operations but also a different order.

A laboratory robot is ideally designed for sampling handling, sample preparation and interfacing sample extracts to HPLC. It is the only technology which performs each of these tasks in an integrated manner (3).

Many assays, especially with newly developed potent drugs, require working with extremely low concentration levels (pcg drug/ml plasma) and therefore high precision. Robotics offers a way to convert research methods into routine methods while increasing the quality, ruggedness and throughput of the assays. Robotics simplifies and guarantees the transfer of assays from laboratory to laboratory where the same robotics systems are employed. Most importantly, robotics separates chemistry from laboratory technique (3). That is, since the laboratory technique of the robot is highly reproducible, poor extraction of samples is usually due to poor optimization of the chemistry of the extraction.

Analytical precision of robotic sample preparation should be improved by eliminating human inconsistencies inevitably introduced in repetitive manual tasks. Each sample is prepared exactly according to the programmed procedure.

With automation, replicate samples, standards and controls can be prepared routinely and at low cost. Several procedures may be programmed for around-the-clock operation, so that sample preparation and instrumental analysis can be more efficiently integrated. Method development is improved through ease of running multiple experiments to optimize conditions and identify error sources.

A robotic system in a development laboratory can be used to set up a variety of unit operations (i.e., extraction, evaporation, etc.) for systematic evaluation. The robot facilitates the conduct of a large number of experiments in a timely manner while controlling the experimental techniques more tightly than achievable manually. In addition, robot systems can have a number of operations being done at the same time, greatly improving efficiency (3).

Results and Discussion

Most HPLC sample preparation procedures fall into one of two categories, component assay or trace analysis. Sample preparation procedures for trace analysis by HPLC are more complicated because a concentration step(s) must be added, usually employing extraction and evaporation steps. Examples of both types will be cited. After the automated sample preparation, the samples can be interfaced to the HPLC in two ways:

1. direct injection - ensures uniform sample history by minimizing sample degradation while held in an autosampler; permits serialized operation,

2. fill autosampler vials - utilizes existing autosampler, does not require HPLC at the robotics area, capable of very small volume injections.

Any new method or technique must be validated before results can be reported, in fact each step or component must be validated. Venteicher and Van Antwerp (4) have reported results on validating various unit operations of a robot system. The Master Laboratory Station (liquids dispenser) was validated for delivering millileter quantities of solvents or reagents. The precision and accuracy were determined by having the three syringes repetitively (X12) deliver 30 ml of three different solutions, 100% water, 25% methanol/water and 100% methanol into a balance containing a tared centrifuge tube. The tube was reweighed following the addition of the 30-ml portions of each respective solvent.

Syringe A -- A precision of 0.04% RSD and an average delivered weight of 30.090 gm $\pm$ 0.025 gm was obtained, (100.3% of theoretical).

Syringe B -- A precision of 0.05% RSD and an average delivered weight of 28.709 gm $\pm$ 0.036 was obtained, (99.6% of theoretical)

Syringe C -- A precision of 0.05% RSD and an average delivered weight of 23,688 gm $\pm$ 0.011 gm was obtained, (99.6% of theoretical).

A robotic hand holding a 1.0 ml syringe was validated using different solvents. Table 2 shows the results of twelve repetitions of drawing 0.5ml of 25% methanol/water into a disposable pipet tip on the syringe hand with delivery to a tared centrifuge tube on a balance. A precision of 0.26% RSD was observed and an average delivered weight of 480.01 $\pm$ 1.25 mg was obtained (99.9% of theoretical).

Samples were then prepared by the robot and the analyte was quantified by an established HPLC method. The overall method precision of 1.0% as observed where the robotic steps of weighing, extraction and subdilution were performed by the robotic system.

TABLE 2

Replicates of the Syringe Hand Delivering 0.5 ml of 25% Methanol/Water

n	wt., mg
1	477.0
2	478.4
3	480.2
4	480.1
5	480.6
6	480.1
7	480.7
8	479.9
9	481.2
10	481.0
11	481.6
12	480.4
Average =	480.01
s =	± 1.25
% RSD =	0.26%

The data are presented in Table 3. Another paper by Hatfield et al (5) also describes results on validation.

To report on the precision of robotic-HPLC results, the paper will describe results by different

types of samples - pharmaceuticals, foods, body fluids and polymers.

Pharmaceutical Assays

Siebert (6) has reported results for oral contraceptive tablets.

TABLE 3

Method Precision of HPLC/UV Analysis with Robotic Sample Preparation

n	Assay, wt., %
1	17.0
2	16.8
3	16.6
4	16.8
5	16.5
6	16.7
$\bar{x}$	= 16.75
s	= ± 0.175
%RSD	= 1.0%

The automation was developed to improve productivity and minimize health hazards by reducing the analyst's contact with steriods. The tablet content uniformity assay involves adding water and chloroform containing the internal standard to the tablets, the tablets are shaken and the active ingredient goes into the chloroform layer, an aliquot of the chloroform layer is transferred to a test tube and evaporated, reconstituted with HPLC solvent using sonication and vortexing for thorough mixing, filtered and pipetted into an autosample vial.

Typical results gave a RSD of 0.67% for norethindrone and 0.93% for ethinyl estradiol. The manual method gave 0.20% and 0.32% respectively. The manual method determined only the instrument (HPLC) precision whereas the automated method incorporated the precision of both the sample preparation and the instrument. No significant difference was observed in the analytical results obtained by means of the automated and manual methods when 3 batches of each product were analyzed by both procedures.

Content uniformity for 2 potencies of anyti-hypertensive tablets by robotic sample preparation has been reported by Walsh et al (7). The procedure is as follows: transfer tablet to a tared tube, weigh tablet, add 25.0 ml of methanol, cap tube, place tube in an ultrasonic bath and then a vortex mixer to disintegrate the tablet quickly, take aliquot and dilute, filter and place in autosampler vial.

The results from 10 tablets from 7 lots is shown in Table 4. One manual determination and two robotic determinations were performed on each lot. For the manual prep, the relative standard deviation on ten tablets ranged from 2.0 - 3.2%. The RSD on a ten tablet assay using the robotic preparation ranged from 1.4 - 3.9%, indicating good precision. The average of the seven lost analyzed were 99.6% and 99.2% of theory for the respective robotic and manual preparations showing the accuracy of the 2 methods is similar. Comparable data was obtained on 50 mg/Tablet assays requiring a secondary dilution step.

A rather complex automated procedure for suppository assays has been reported by Hatfield, et al (5). The procedure is described in Table 5. The method validation consisted of preparing three lots

of suppositories for content uniformity comparison and the results are shown in Table 6. The validations for the suppository assay show the robotic system to be accurate and precise in its measurements and demonstrated equivalency between robotic and analyst sample preparation.

TABLE 4

Comparison of Robotic and Manual Preparation Content Uniformity Tests, Anti-hypertensive Tablets (25 mg/Tablet)

Lot	Range (% of Theory)	Average (Mg/T)	% RSD (10)	(% of Theory)	Average (Mg/T)	% RSD (10)
1	96.1-106.0	25.2	3.2	98.0-107.3	25.4	2.5
				97.0-101.6	24.8	1.4
2	94.2-100.2	24.3	2.0	93.4-103.2	24.9	2.9
				94.1-102.4	24.4	2.4
3	94.1-102.4	24.8	2.5	98.2-104.9	25.1	2.4
				97.6-102.8	25.0	1.6
4	94.1-103.2	24.5	2.5	91.5-103.8	24.5	3.9
				94.6-100.9	24.7	2.0
5	96.9-102.5	24.8	2.0	95.9-105.4	24.7	3.1
				95.8-102.7	24.9	1.9
6	96.0-101.8	24.7	2.0	96.1-106.9	25.0	3.2
				95.2-105.4	24.9	3.2
7	96.4-104.4	25.2	3.0	93.3-104.5	25.1	3.1
				99.6-109.2	26.0	2.7
Grand Average = 24.8				Grand Average = 24.9		

Commpendial Requirements
(Uniformity of 10 tablets): 1) No value outside the range of 85.0-115.0% of label claim
2) $RSD_{(10)} \leq 6.0\%$

An analyst can prepare one batch of suppositories for

content uniformity assay (10 samples) in one day.

TABLE 5

Unit Operations for MONISTAT Suppository Assay

- WEIGH SUPPOSITORY

- DISPERSE SUPPOSITORY
  Add 30 mL Pentane
  Vortex and Centrifuge
  Withdraw Pentane

- EXTRACT EXCIPIENT MATRIX (repeat 4 times)
  Add 20 mL Pentane
  Vortex and Shake
  Centrifuge and Withdraw Pentane

- EVAPORATE RESIDUAL PENTANE

- RECONSTITUTE SAMPLE
  Add 40 mL Methanol
  Sonicate and Shake
  Sonicate and Vortex

- PIPET SAMPLE ALIQUOT TO SECOND TUBE

- EVAPORATE TO DRYNESS

- ADD INTERNAL STANDARD
  Vortex and Sonicate

- FILL CAPPED VIALS USING SYRINGE HAND

The robotic system prepares three batches in the same twenty-four hour period. Not only is the robotic system freeing the analyst to perform other duties,

but considering the lab receives over one hundred batches of suppositories a year, the productivity gains and time savings are large.

TABLE 6

MONISTATE Suppository Assay - Robot vs. Analyst

	ROBOT	ANALYST
	% LABEL CLAIM	
Content	101.7	100.6
Uniformity	105.2	105.1
	99.1	99.6

Composite tablet samples have been prepared using robotics with a typical batch of ten samples consisting of 5 to 32 tablets per sample (8). The system weighs the tablet, adds diluent, homogenizes the sample to disintegrate the tablets, dilutes the sample when necessary, removes and filters replicate aliquots, dispenses each aliquot into an HPLC vial and caps the vial. Methods were validated for Lanoxin 0.125 mg and 0.250 mg tablets and Sudafed 30 mg and 60 mg sugar-coated tablets. The system was designed with flexibility in mind not to limit its application to a single product and the system is not dedicated to a particular HPLC system or analytical technique. Development of new procedures can proceed in parallel with production related sample preparation. Table 7 shows results for Lanoxin and Table 8 for Sudafed tablets.

The robotic system prepared composite Lanoxin and Sudafed tablet samples with results equivalent to those prepared manually be established procedures.

For typical batches of 10 samples, the occupied analyst time related to sample preparation (4.5 hours of Lanoxin and 2.5 hours for Sudafed was reduced to 45 minutes. Additional advantage of the Sudafed system was a 90% reduction in solvent consumption and elimination of duplicate sample preparation.

TABLE 7

HPLC Assay Reproducibility for Lanoxin Tablets Automated and Manual Preparation Percent Label Strength by Peak Area

	0.125 mg		0.250 mg	
Sample	Manual	Automated[1]	Manual	Automated[1]
1	99.1	97.3	98.6	98.3
2	98.0	96.8	100.4	98.8
3	97.9	98.0	99.8	98.9
4	97.5	99.0	100.2	99.6
5	98.1	98.0	100.8	98.7
6	98.0	99.0	99.3	99.0
7	96.3	97.7	98.2	97.9
8	97.9	98.6	99.8	100.0
9	98.1	98.7	100.4	100.3
mean	97.9	98.1	99.7	99.0
Std. Dev.	0.729	0.769	0.871	0.780
%RSD	0.745	0.784	0.874	0.787
Range	2.8	2.2	2.6	2.0

[1] Values corrected for diluent displaced by sample.

Greenbergh et al (9) have transferred an existing manual composite assay to a totally

automated robotic method. The robotic method eliminates the large consumption of solvents and multiple dilutions previously required.

TABLE 8

HPLC Reproducibility for Sudafed S/C Tablets Automated and Manual Preparation Percent Strength by Peak Area

	30 mg		30 mg	
Sample	Manual	Automated[1]	Manual	Automated[1]
1	95.8	93.9	99.7	101.0
2	97.2	97.4	99.7	99.9
3	95.8	96.7	101.0	100.6
4	96.4	97.5	101.3	99.6
5	96.9	98.5	102.2	100.4
6	95.9	98.1	99.8	102.5
7	97.2	96.8	98.9	102.2
8	96.9	95.2	99.2	99.9
9	98.3	97.0	100.3	99.8
10	96.8	95.9	99.7	101.2
11	96.1	97.4	99.6	102.6
12	97.4	94.8	99.7	99.9
13	95.4	98.9	100.3	99.2
14	95.7	97.8	99.4	99.2
15	98.3	97.9	99.2	102.0
mean	96.7	96.9	100.0	102.0
Std. Dev.	0.910	1.41	0.891	1.17
% RSD	0.941	1.46	0.891	1.16
Range	2.5	5.0	3.4	2.8

[1]Values corrected for diluent displaced by sample.

TABLE 9

Manual Recovery Studies for Composite Assay

Nominal Composite Dosage (mg)	mg Added	mg Recovered	% Recovery
850	846.49	860	103
	846.92	862	102
	843.27	863	102
	857.70	875	102
	853.59	875	103
1130	1123.88	1140	101
	1183.14	1210	102
	1107.46	1130	102
	1126.61	1150	102
	1133.95	1150	101
1700	1728.70	1730	100
	1684.17	1700	101
	1709.10	1730	101
	1696.96	1710	101
	1704.31	1720	101
	1703.92	1720	101
2300	2330.02	2340	100
	2331.12	2330	100
	2309.83	2310	99.8
	2309.67	2310	100
2875	2882.18	2860	99.2
	2917.41	2910	99.7
	2857.43	2830	99.2
	2861.51	2830	99.0
	2859.28	2840	99.2
	2865.80	2850	99.4
			$\bar{x}_{26}$ = 101
			RSD = $\pm$ 1.19

The data for the manual version of the method is shown in Table 9 and the automated method in Table 10. A diagram of the assay is shown in Figure 1.

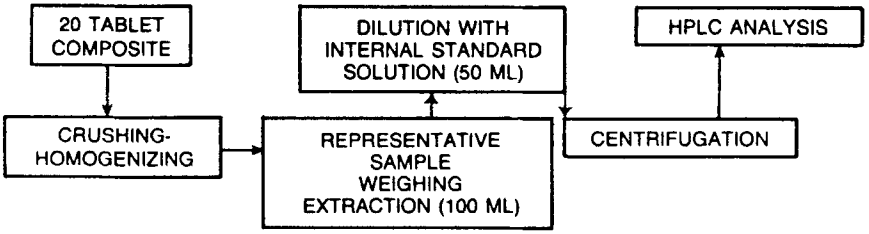

Figure 1. Diagram of an automated stability indicating composite assay.

Synthetic samples were used in both the manual and robotic recover studies, and peak areas were employed for quantitation. Excellent agreement was found between the manual and automated methods.

Dosage formulation studies for toxicology analyses involving dosed feed blends has been reported by Rollheiser et al (10). The layout of the robotic system for this assay is shown in Figure 2. The components analyzed were methyleugenol (a flavoring agent), disulfiram (an alcohol deterrent), and di-(2-ethylhexyl)phthalate (a plasticizer) also known as DEHP.

The results in Table 11 are for triplicate analyses of 'grab' samples taken from right, left and bottom locations of a twin shell blender. The method includes weighing, liquid-liquid extractions, centrifugation, dilution and HPLC injection and analysis. The HPLC results, at the 95% confidence level, indicated that for methyleugenol, the

precision of the robot method is better than that of the equivalent manual method and equivalent to the precision of the standard manual method.

TABLE 10

Robotic System Recovery Studies for Composite Assay

Nominal Composite Dosage (mg)	mg Added	mg Recovered	% Recovery
850	853.1	858	101
	848.2	852	100
	853.2	857	100
	852.5	846	99.2
	850	847	99.6
1130	1131.8	1140	101
	1129.1	1120	99.2
	1130.0	1160	103
	1130.6	1110	98.2
	1134.0	1140	101
1700	1703.1	1700	99.8
	1702.7	1650	96.9
	1704.2	1680	98.6
	1699.6	1670	98.6
2300	2302.4	2276	98.9
	2301.8	2230	96.8
	2302.4	2254	97.9
	2303.3	2293	99.6
2875	2877.6	2890	100
	2876.7	2860	99.4
	2875.0	2880	100
	2872.2	2900	101
	2876.7	2940	102
		x 23 = 99.6	
		RSD = ±1.50	

For disulfiram and DEHP, the precision of the robotic method is not significantly different from that of either the equivalent manual method and similar to the precision of the standard manual method.

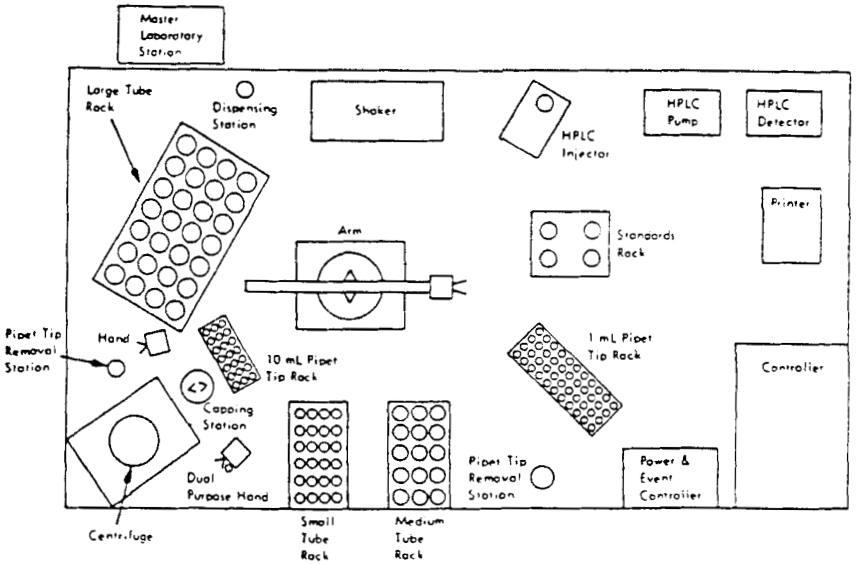

Figure 2. Table layout for robotic analysis of dosed feed.

For all three chemicals, the accuracies of the three methods were equivalent. The standard manual method uses larger volumes of solvents where the manual method uses the same volume of solvents as the robotic method.

Food Assays

Hurst et al (11) have reported on the determination of carbohydrates in mild chocolate and theobromine and caffeine in cocoa.

TABLE 11

Automated Homogeneity Evaluation by High Performance Liquid Chromatography

Chemical	Sample Location	Automated Found (%)	SD[a]	Simulated Found (%)	SD[a]	Manual Found (%)	SD[a]
Methyleu-genol	Right	98.0	1.0	97.0	1.0	96.7	0.7
	Left	98.5	0.4	96.0	2.0	96.7	0.2
	Bottom	98.5	0.4	95.9	2.4	96.2	1.0
	-	----	---	----	---	----	---
	x	98.3	0.6	96.3	1.7	96.5	0.7
Disulfiram	Right	100.1	1.4	101.3	2.5	99.8	0.4
	Left	100.0	1.5	97.4	2.0	100.7	1.5
	Bottom	101.1	1.5	97.7	1.4	99.3	0.8
	-	-----	---	----	---	-----	---
	x	100.4	1.4	98.8	2.6	99.9	1.1
DEHP	Right	98.5	1.3	98.6	0.3	98.3	0.4
	Left	97.7	1.0	97.9	0.7	97.8	0.3
	Bottom	96.9	1.0	96.2	1.6	96.5	0.3
	-	----	---	----	---	----	---
	x	97.7	1.2	97.6	1.4	97.5	0.9

[a]Standard Deviation

TABLE 12

Comparison of Manual and Robot Prepared Samples for the HPLC Determination of Carbohydrates (n=5)

	Manual	%Cv	Automated	%CV
% Sucrose	44.30	5.24	43.94	6.13
% Lactose	8.01	7.34	8.10	8.94

The method consisted of weighing, diluting to a calculated volume, mixing, heating filtering and injecting into an HPLC. The results are shown in Tables 12 and 13.

The automation of these two procedures produced data similar to that from manual preparation schemes. The analysis time was about the same for the manual or robot method. In these analyses, the heating period created "dead time" for the analyst as the time was to short to allow accomplishments of the other tasks.

TABLE 13

Comparison of Manual and Robot Prepared Samples for the HPLC Determination of Theobromine and Caffeine (n=5)

	Manual	%Cv	Automated	%Cv
% Theobromine	2.54	2.43	2.46	3.47
% Caffeine	0.19	3.16	0.23	3.18

To be adaptable to the robot, the methods were scaled down to use smaller volumes of solvents. Pilot studies showed no loss of precision or accuracy using the syrup and cocoa matrices.

Dykutzy (12) has reported on automated caffeine analysis of tea leaves. The method consists of weighing the sample, adding reagents and solvents, heating the mixture, adding water back to initial volume after the heating, mixing and allowing to cool, taking aliquot, filtering and injecting into HPLC. The results from replicate samples are shown in Table 14 and indicates good agreement between the

manual robot method.

Automated cleanup and analysis of food samples (cereal, meats, etc.) for vitamin assay by HPLC has been reported by Higgs et al. (13) The automated cleanup procedure is described in Figure 3.

TABLE 14

Comparison of Robotic and Manual Procedures - Percent Caffeine

n	Robotic Method	Manual Method
1	2.98	2.76
2	2.81	2.80
3	2.71	2.68
4	2.78	2.76
5	2.92	2.75
6	2.95	2.68
7	2.79	2.78
8	2.89	2.84
9	2.92	2.85
average	2.86	2.77
s	±.09	±.06

The results for three different types of samples are shown in Table 15. The results are comparable (agreement to within 10%) with coefficients of variation less than 20% even for samples low in thiamine content. In this assay, the extraction and clean-up procedures for the analysis of Vitamin B_6 and thiamine in foods are essentially identical so one extracted food sample can form the basis for the simultaneous analysis of both vitamins. The robot can be programmed to inject an aliquot of sample into each of two HPLC analytical systems. Since both the Vitamin B_6 and thiamine procedures use internal

standards, which are added to the sample prior to analysis, it is not necessary to monitor the various volume dilutions that may occur during the analytical steps.

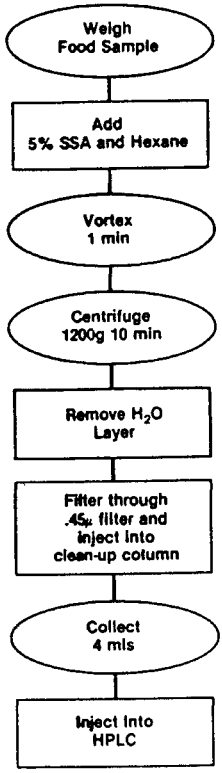

Figure 3. Block diagram for the robotic extraction of thiamine from food samples.

Polymer Analysis

Automated sample preparation of polymeric materials for Size Exclusion Chromatography (SEC) has been published by Klinger (14). These samples included liquid latex dispersions, powdered resins,

compounded pellets, thin films and sections of test specimens or from finished molded applications. The automated steps are weighing, adding solvent, shaking, filtering and pipetting into an autosampler vial.

TABLE 15

Comparison of Manual and Robotic Extraction Procedures for Thiamine

	Manual	Robotic
	mg/100g	
CHICKEN MEAT, Skinned		
Breast	.049 ± .004	.053 ± .002
Leg	.062 ± .009	.061 ± .012
Thigh	.050 ± .004	.053 ± .013
CEREAL, bran	2.02 ± .127	2.55
POLLEN	.763 ± .067	.701 ±.060

The method was validated by loading appropriate autosampler vials with aliquots from an accurately prepared master solution of a wel-characterized polymeric sample. The manual and automated procedures were next compared by preparing a series of samples of the same polymer by the most qualified laboratory analyst versus the Zymate System. The results are shown in Table 16.

Data for the single master solution showed the analytical SEC measurement technique to have a relative standard deviation or coefficient of variation (c.v.) of 0.7%. Meanwhile, data for the individual analyst prepared samples showed a c.v. of

TABLE 16

Precision of SEC Sample Preparation Procedures

Autosampler Vial Number	Single Master Solution	Individual Analyst Prepared	Individual Robot Prepared
1	6268722	6046423	6185122
2	6149889	5736456	6204019
3	6216656	6161406	6174092
4	6217949	56443106	6235229
5	6238351	6002643	6078786
6	6192560	5967837	6181850
7	6248978	6110184	6295481
8	6261249	6019405	6312532
9	6232278	5950841	6239766
10	6245957	6021112	6129080
11	6130128	6147072	6189439
12	6196419	6064400	6120315
13	6167603	5747900	6061738
14	6212808	5964464	6244487
15	6251851	6303961	6236012
16	6153409	6133559	6244590
17	6204324	5899903	6156511
18	6187714	5984285	6156511
19	6154370	5998073	6047415
20	6155822	6068795	6106739
21	6162133	6151056	6011993
22	6169464	5849034	6166541
23	6169115	6357655	6108912
24	6138396	5971181	6147558
Mean	6196922	6012531	6168092
Std Dev	42078	165223	76695
C.V. (%)	0.7	2.7	1.2

2.7% and finally, data for the individual robot prepared samples exhibited a c.v. of 1.2%. The robot procedure shows improved precision over the manual method.

Blood Fluids Assays

Schoenhard (3) has reported on automated assays for drugs in animal and human plasma.

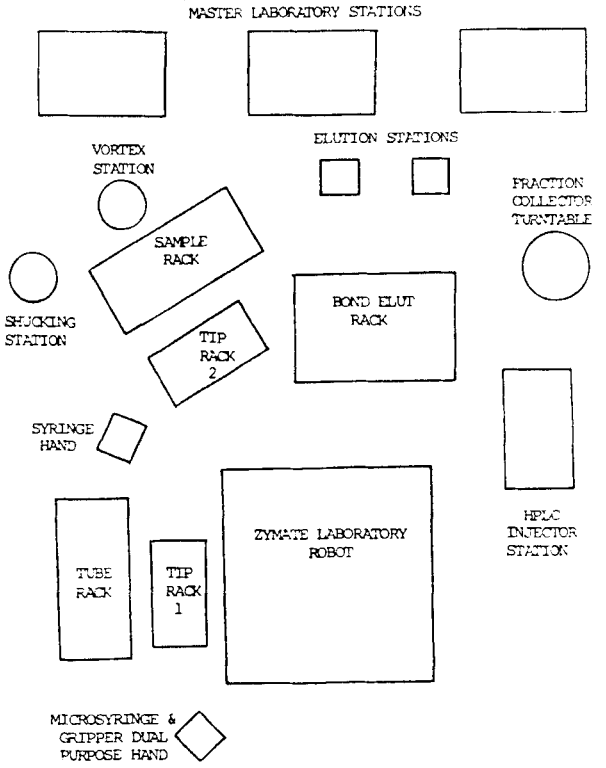

Figure 4. Table layout for robotic assays for drugs in animal and human plasma.

The robotic method is used for sample preparation and

to interface to HPLC prior to quantitation by RIA. A layout of the system is shown in Figure 4. The automated method takes aliquots of the samples, adds ion suppressing reagents, mixes the sample, activates the solid phase extraction column, adds the sample to column, selectively elutes the drugs into a carousel for evaporation and injection into HPLC.

The coefficient of variation for the combined robot steps of sample handling, sample preparation and interfacing to the HPLC is 1.45% (N=13). This high degree of precision obtained by the robot method is *one order* of magnitude better than obtained manually. Furthermore, the quality is maintained when the method is conducted routinely. In addition, this precision is a necessary condition in order to develop an assay with a detection limit at the low pg/ml plasma concentration of the drug. A big improvement came from automating the solid phase extraction, because the flow rate was very reproducible resulting in more reproducible kinetics with the robot versus the manual method .

Lewis et al (15) has reported the analysis of drugs in biological samples from small laboratory animals. These assays of 0.5 ml of plasma or less does not permit a second sampling if the original sample is consumed in an unsuccessful analysis. Furthermore, the method must measure plasma concentrations over four or five orders of magnitude from high pg/ml to low ng/ml level. The method utilizes liquid-liquid extraction which is labor intensive and prone to variable losses of analyte. A flow chart of the method is shown in Figure 5.

Improved precision was one of the expectations of robotic automation of liquid-liquid extraction procedures. The results using external

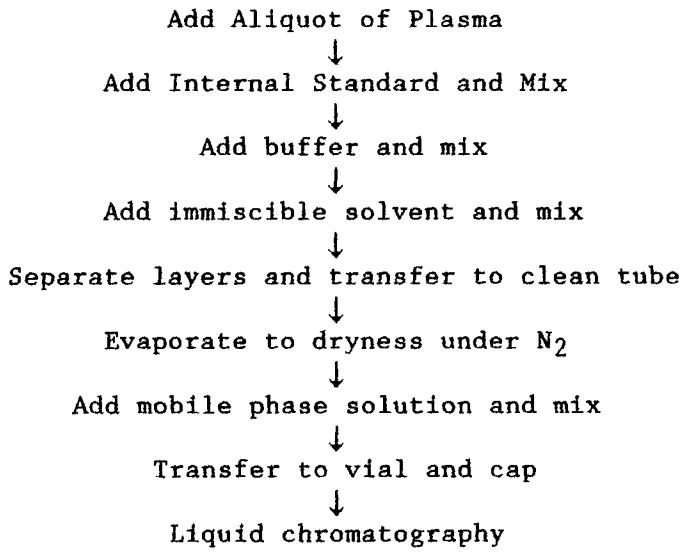

Figure 5. Flowchart of unit operations for chromatographic analysis of plasma with prior liquid-liquid extraction.

TABLE 17

Comparison of Relative Precision of Overall Analysis at Three Concentrations of Analyte Using Measured Extraction Volumes and External Standardization

Plasma Concentration	CV Manual	(N)	CV Robot	(N)
(ng/ml)	(%)		(%)	
20	24.1	(14)	13.6	(7)
200	6.7	(14)	3.9	(11)
1000	5.8	(12)	3.2	(6)

standardization for both the manual and robotic procedures are given in Table 17. The precision deteriorated at the low levels because precision of the analytical measurement (HPLC) was significant with respect to the precision of the sample preparation (robot).

These results show how robotic automation of multi-stage sample preparation procedures such as liquid-liquid extraction can improve precision by approximately twofold or better. Improved precision can also produce improved accuracy for individual samples with an appropriate standardization protocol. An analyst with the assistance of a robot could product 80 samples/day whereas an experienced analyst could only prepare 40 samples/day.

Myers (16) has reported on assays of theophylline and tolazamide from serum samples. The automated procedure preconditions a solid phase extraction column, adds sample and internal standard to the column, elutes the compound of interest from the column, and places it in an autosampler vial.

The average within-day coefficient of variation is 4.25%, compared to 8.38% when processed by an experienced chemist.

In these assays, the robot system required 2 hours to process 50 samples, whereas a chemist requires 4 hours.

Laboratory robots have been used extensively to automate many HPLC procedures to give better quality data while bringing a new level of automation resulting in substantial gains in productivity.

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