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Economic approach to robotic sample pretreatment in high-performance liquid chromatography

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Abstract

Economic automation of multistep analytical sample preparation is still a largely unsolved problem. Existing systems either handle only a few steps of a complex analytical scheme or they offer a complete solution at relatively high cost. This paper shows, using a rather general example that it is possible to construct a system in the analytical laboratory with an optimal balance between automating most steps of the analysis scheme and still keeping the costs of the system within bounds. A practical example from pharmacokinetic analysis is presented; pretreatment of blood plasma samples for nifedipine determination by high-performace liquid chromatography. The automated method involves steps such as aliquoting, pH adjustment, liquid–liquid extraction and evaporation to dryness. The system is built around a robotic arm from commercial equipment not designed originally for use with a robot. Analytical results and costs are compared to the manual method.

Keywords: Sample handling; Automation; Nifedipine

1. Introduction

In recent years it has become commonplace that the bottleneck in routine analytical work is sample preparation. Simple tasks like aliquoting, pH adjustment, liquid–liquid or solid-phase extraction need to be carried out by laboratory personnel on a large number of samples. The dull repetitive character of this work often results in unnoticed operator errors that compromise the overall quality of the final results.

Several important developments in laboratory automation can solve these problems in certain types of laboratories. In clinical laboratories virtually full automation has been achieved. This obviously required enormous efforts in the engineering of the respective instruments and chemistries. The great input was justified by the large number of samples for clinical analysis.

Non-clinical analysts rarely encounter a similar situation. Their tasks are more variable and the number of samples per procedure is less. For the general analytical laboratory there have been other options available for automation. Instruments used

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for repetitive analyses like chromatographs and atomic absorption spectrometers are frequently equipped with automatic samplers. Serial pipetting and dilution can conveniently be done by so-called automatic sample processors. Yet autosamplers and automatic sample processors automate only one or two steps of a full analysis scheme. Many typical schemes consist, however, of about a dozen steps. These may include liquid–liquid and solid-phase extraction and other steps that cannot be easily automated with the mentioned devices.

The automation of a typical multistep sample preparation process poses a delicate strategic question. With present-day technology it is no problem to automate even the most complicated procedure. The problem is merely economic: how much cost is justified to solve a particular problem. The mechanical and electronic development required is typically made by instrument manufacturers. They must, however, ensure a great turnover of the equipment. This means that the sample pretreatment device has to be fairly general, i.e., easily adaptable to many different analytical schemes. This, in turn, makes the equipment rather complex and expensive. The resulting high costs make this kind of equipment unavailable (economically not justifiable) to most laboratories. This vicious circle appears to explain that most analytical laboratories still have to struggle with manual sample preparation.

In this paper we suggest a scheme whereby the costs and the benefits from automation are better balanced. What we propose is not a ready solution for every problem; rather, we present using a real example, how useful compromises can be made to achieve a good balance between costs, efforts and results. Nevertheless, many of the technical details shown below might be worth reproducing.

The essential part of our system is a commercially available robotic arm. While the cost of such a device is typically less than that of a basic HPLC or GC system, it does not immediately replace the laboratory technician. The robot cannot handle most of the tools of the trade, e.g., pipettes, burettes, etc., and even if it can (e.g., analytical balance) it does so all too slowly.

To circumvent this problem, manufacturers have built robotic workstations with all sorts of specialised mechanical units surrounding and assisting the robot [1–6]. As mentioned above, such systems are capable of full automation of varied tasks but they are expensive due to the use of dedicated units, produced in small numbers. Not surprisingly the majority of recent reports about robotic sample pretreatment [7–16] come form large, mainly pharmaceutical companies.

Articles appearing about automated analysis systems in the scientific literature deal mainly with technical matters and apparently are not concerned with cost efficiency. In contrast to this a recent representative survey [17] with some of the best experts of this field has revealed a marked slowdown of development and this has been attributed at least partly to the problems of cost efficiency.

2. Strategy of automation

Based on the previous statements our strategy involved the following steps: (i) purchase only the basic robotic arm; (ii) adopt – if this can be done easily – general laboratory equipment to assist the robot; (iii) construct some small parts like fingers for the robot dedicated to this generic environment and (iv) do not attempt full automation by all means (and costs); a small amount of manual interaction may be justified.

This strategy requires that the analytical laboratory: (1) is willing to invest energy into system development; (2) has access to a mechanic (internal or contract) during system development and (3) can assign a laboratory technician to oversee and assist the robot intermittently.

Before turning to the details it is noted in advance that we have made large series of analyses both before [18] and after the robotization of the same analytical procedure. We found that our work became much easier while the quality of the results of a difficult trace analysis in blood did not suffer, as will be shown below.

3. Experimental

3.1. Reagents

Nifedipine [dimethyl-1,4-dihydro-2,6-dimethyl-4-

(2-nitrophenyl)-3,5-pyridine dicarboxylate] and the internal standard [dimethyl-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate] were supplied by EGIS Pharmaceuticals (Budapest, Hungary). Methanol, hexane and dichloromethane were purchased from Romil Chemicals (Loughborough, UK). NaOH was a product of Fluka (Buchs, Switzerland), acetic acid was supplied by Soprelec (Evry, France).

All chemicals were of analytical grade. The solutions were prepared with doubly distilled water. Calibrations were obtained with human plasma from EGIS Pharmaceuticals containing CPD as anticoagulant. Plasma samples were stored at −20°C.

3.2. HPLC analytical system

Separation of nifedipine was carried out on an ODS Hypersil (Hewlett-Packard, Palo Alto, CA, USA) 5 μm analytical column (200×4.6 mm I.D.) with a BST ODS Hypersil pre-column (5 μm, 20× 4.0 mm I.D., Bio Separation Technologies, Budapest, Hungary). Column switching was used to eliminate long retention time components of the plasma. The eluent was methanol–0.01 mol/l acetate buffer, pH 4 (75:25, v/v). It was delivered by an LKB 2150 (Pharmacia LKB, Bromma, Sweden) and a Beckman 114 M (Beckman Instruments, Berkeley, CA, USA) HPLC pump with a flow-rate of 0.8 ml/min. Detection was done with an electrochemical detector (BAS LC-3C, equipped with a BAS LC-44 thin-layer cell [Bioanalytical Systems, West Lafayette, IN, USA)] at a glassy carbon electrode at 1000 mV vs. Ag/AgCl electrode. A 50 μl sample was introduced into the chromatograph via an automatic injector. Column switching was accomplished with another automatic injector. Injector switching, data acquisition and evaluation were controlled by a 486 AT IBM compatible computer using Borwin 1.20 chromatography software (JMBS Developpement, Le Fontanil, France). For quantitative evaluation the internal standard method was applied choosing dimethyl-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine dicarboxylate, a compound very similar in structure to nifedipine, as the internal standard. The peak height ratio of nifedipine to internal standard was used for quantitation.

3.3. Sample preparation procedure

The steps for extracting and measuring the nifedipine content of plasma samples are described in Fig. 1. In the present work steps 2–6 and 8–9 were carried out by the robot, while steps 1, 7 and 10–12 were done manually. (The reasons for using manual redissolution/injection will be explained in Section 3.4.7 later.) Step 13 is controlled and carried out by the computer. Steps 1–6 are carried out consecutively on individual samples of a batch, then the whole batch is centrifuged together.

3.4. Robotized system for sample preparation

We have slightly modified commonly available instruments to fit to the robot. In the following sections the components of the robotized system will be described.

3.4.1. The robot

We used a Mitsubishi Electric "Movemaster" type RV-M1 five axis robot which mimics the movements of a human arm. The programs for the robot were written in Borland C using a simple set of special commands which is interpreted by the robots microprocessor unit.

To ensure easy and safe gripping and handling of test- or centrifuge tubes, two different pairs of fingers were designed and fabricated by us. These could be used alternatively by rotating the wrist by 180°. One pair of fingers was designed to grip cylindrical objects, and was made of stainless steel equipped with two pairs of plastic cylinders fastened with screws. The other pair of fingers was made by modifying a laboratory forceps. This pair was used to lift and move test tubes. The tips of the forceps were covered with silicone rubber tubes. The tips were inserted in the closed position into the mouth of the dry test tube to be handled. When opened, they adhered to the inside of the tube and thus they could lift and handle the tube. Care was taken that the forceps fingers touched only the upper dry portion of the test tube to avoid cross-contamination.

3.4.2. Liquid dispensing units

A Radelkis (Hungary) OP-930 type automatic burette was used for dispensing larger (>200 μl)

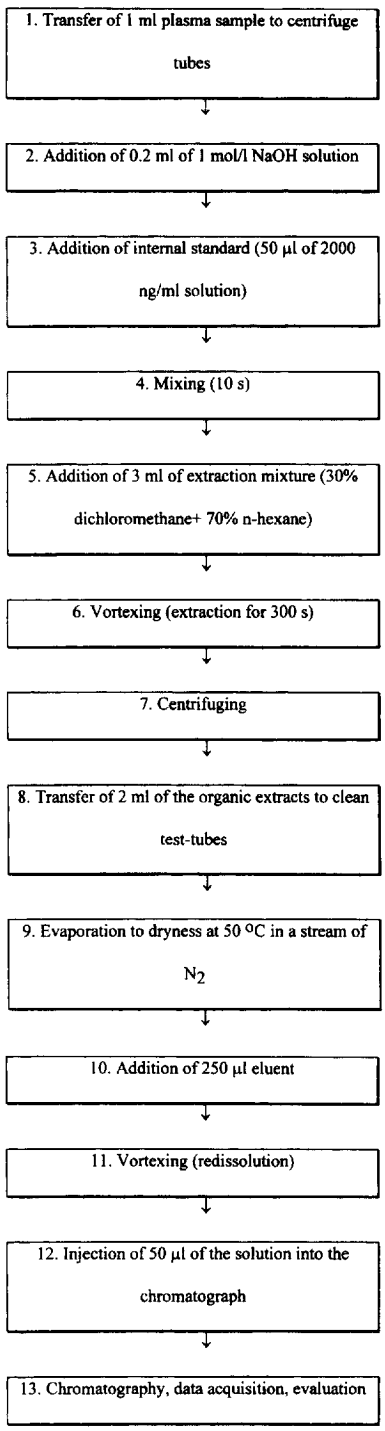

Fig. 1. Flow diagram of the robotic sample preparation steps.

volumes of liquids. The electronic part of this fairly old design had to be modified to permit control of both the filling and dispensing operations by computer, including adjustment of speed and direction of piston motion, and the volume of liquid dispensed. The burette has two cylinders, allowing handling of two liquids. One cylinder was used for aqueous solutions, and the other for organic solvents. Both cylinders were connected by a PTFE tube (internal diameter 1 mm) to injection needles for dispensing. The needles were equipped with PTFE sleeves for easy handling by the robot.

For dispensing small volumes (10–200 µl) of liquid, a Metrohm (Switzerland) 665-Dosimat type automatic burette was used. This intelligent instrument could be linked to the computer via an RS232 (series) interface, and it was easily programmed using its own set of commands. The Metrohm burette was used for dispensing of internal standard and nifedipine solutions, respectively. The burette was connected by a PTFE tube to the barrel of a microliter syringe. The syringe was equipped with a PTFE sleeve for easy gripping by the robot fingers.

3.4.3. Racks for test-tubes

The safe and accurate handling of test- and centrifuge tubes is very important and requires attention in the design of the racks and of the robot fingers.

Racks were fabricated from Plexiglass to hold test- or centrifuge tubes. The racks were designed to accommodate the highest possible number of tubes within the work envelope of the robot, which is surprisingly limited. In consequence of the close packing of the tubes it was necessary to design and fabricate forceps-type fingers by which the tubes could be gripped from the inside.

A Plexiglass rack was made for the solution dispensing needles, and another one for the ground-glass stoppers of the centrifuge tubes.

3.4.4. Test-tube thermostat

After extraction of the analyte from the plasma samples, the organic phase is evaporated to dryness at 50°C in a stream of N_2. An MTA-Kutesz (Hungary) 615 type test-tube thermostat was modified for this purpose. The thermostat has cylindrical holes for the test-tubes in a heated aluminium block. A dark

Plexiglass lid was fabricated for the test-tube thermostat to protect samples from light and dust. Injection needles 60 mm in length were mounted in the lid for N_2 introduction over the samples during evaporation. The vapours were removed by a blower built into the side of the lid. The lid was opened and closed by the robot.

3.4.5. Centrifuge tubes, vortex mixer

Glass centrifuge tubes with conical bottom and ground-glass stopper were specially designed and made with a volume of about 6 ml for the robotic system. For safe gripping by the robot, the tops of the stoppers were covered with a thin layer of silicone rubber.

A Heidolph Reax 2000 type vortex mixer was used for homogenising solutions and carrying out extraction.

3.4.6. Control unit

The robot system was run by an IBM-AT 386 compatible computer. The interface was an Advantech PCLAB PCL-812PG card with 16 channel A/D and two channel D/A converter. The drive unit of the robot was connected to the computer through a parallel (Centronics) interface. The Metrohm burette was controlled through a serial (RS232C) port. All other control signals (for the Radelkis burette, thermostat, N_2 valve) were supplied by the PCLAB card. The programming language was Borland C.

3.4.7. Injection with robot

The redissolution of dried sample extracts and their injection into the HPLC system (i.e., steps 10–12 in the sample preparation scheme) have also been automated using the robot. However this option was not used when a large number of samples had to be analysed. We have found that dividing the robot's "attention" between sample pretreatment and HPLC injection had slowed down the robot substantially. This was due to the conflicting time schedules of the two procedures. In these instances injection was carried out manually. The sample throughput could be doubled by allowing manual injections. (In laboratories where the HPLC system is equipped with an autosampler the robot might redissolve the dried extracts and transfer them to the autosampler.)

4. Results and discussion

4.1. Analytical results

The performance of the robotic sample preparation system was checked by validating it for human plasma measurements of nifedipine.

Three chromatograms are shown in Fig. 2, one obtained with nifedipine dissolved in the mobile phase, one with blank plasma and the last one with a spiked plasma sample.

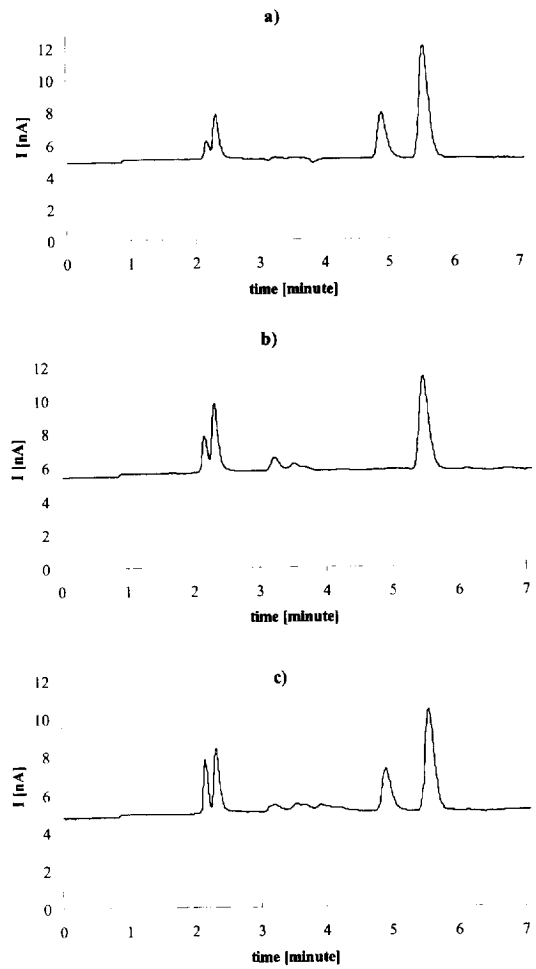

Fig. 2. (a) Chromatogram of 20 ng/ml nifedipine and 100 ng/ml internal standard dissolved in the mobile phase. (b) Chromatogram of a plasma sample containing 100 ng/ml internal standard. (c) Chromatogram of a spiked plasma sample containing 20 ng/ml nifedipine and 100 ng/ml internal standard. For chromatographic conditions see Section 3.2.

Table 1
Inter-day precision of the measurement with manual and robotic sample pretreatment at different nifedipine concentration levels

Nominal concentration (ng/ml)	Calculated concentration mean±S.D. (ng/ml)		R.S.D. (%)	
	Manual (n=6)	Robotic (n=6)	Manual	Robotic
2.5	2.8±0.2	2.5±0.2	8.7	6.5
5	5.5±0.4	5.1±0.6	6.6	12
10	10.6±1.7	10.0±0.5	16	4.5
20	18.6±1.2	20.3±1.3	6.7	6.6
30	29.1±1.4	29.9±2.3	4.8	7.7
50	50.9±3.1	49.6±3.5	6.2	7.1

The calibration curve was constructed from parallel calibration measurements on six different days. The equation of the calibration curve obtained by fitting with the least squares method is $y=0.0213x-0.0133$ ($R^2=0.9963$).

From the calibration measurements the inter-day precision of the method was determined. The calculated average concentrations and relative standard deviations found with six parallels at six different concentration levels are listed in Table 1.

The intra-day precision was determined from six parallels at three concentration levels. Data are shown in Table 2.

For comparison of manual and robotic sample preparation spiked human plasma samples were analysed. Nine plasma samples containing 20 ng/ml nifedipine were pre-treated manually and another nine using the robotic system. From the nifedipine to internal standard peak height ratios the concentration of the samples was calculated based on the calibration curve.

The precision of the measurement with robotic sample preparation was 2.13%. It did not differ significantly from that of the manual sample preparation, which was 3.29%. In fact these values for the total analysis did not exceed that of the chromatographic system alone at the same concentration level (3.26%). This means that the contribution of the random error of the sample preparation to that of the total measurement was insignificant.

The accuracy was calculated from the percentage difference of the nominal and measured concentration. The average accuracy values for the robotic and the manual procedure are also very close, 4.81% and 3.96%, respectively. This indicates that no further determinate errors are introduced via the robotic sample preparation system.

Percentage recovery from the liquid–liquid extraction was determined by dividing the peak height of nifedipine (or internal standard) obtained for extracted plasma sample by the peak height of a directly injected aqueous standard and multiplying by 100. Recoveries were determined at nifedipine concentrations of 10, 30 and 50 ng/ml. The concentration of the internal standard was 100 ng/ml in each case. The recovery obtained for nifedipine at 10, 30 and 50 ng/ml was 85%, 90% and 89%, respectively. The recovery of the internal standard in the same measurements was 86%, 87% and 85%. There was good agreement in the recovery of

Table 2
Intra-day precision of the measurement with robotic and manual sample pretreatment at three different concentrations of nifedipine

Nominal concentration (ng/ml)	Manual (n=6)		Robotic (n=6)	
	Calculated concentration (ng/ml)	R.S.D. (%)	Calculated concentration (ng/ml)	R.S.D. (%)
5	4.7±0.3	7.0	5.3±0.3	5.5
20	18.0±1.3	7.0	21.2±0.4	1.7
50	48.4±1.7	3.5	53.2±1.3	2.3

nifedipine and the internal standard. The detection limit of the analytical method is 0.45 ng/ml. The limit of quantitation is 1 ng/ml nifedipine in plasma (the lowest concentration measured in the calibration, where the R.S.D. is still less than 20%).

Taking all matters into consideration it can be established that the analytical performance of the robotic system is at least as good as that of the highly skilled operators in the manual method (all Ph.D. analytical chemists). Other advantages (i.e., the system operates in the dark continuously, plasma samples need not be treated excessively by hand, human error is excluded to a large extent, tiring manual work is avoided) make it far superior to the manual method.

4.2. General assessment of the robotic system

The robotic system described in this paper has been in almost continuous use for more than a year now. Many pharmacokinetic analyses on plasma samples have been carried out with it [19]. The results have justified the strategy explained in introduction.

The analytical procedure automated in this work consists of many steps and it is typical for a variety of procedures. It includes sample aliquoting from a liquid sample, addition of a pH adjusting solution, addition of internal standard, mixing, liquid–liquid extraction, phase separation, evaporation to dryness and reconstitution in HPLC eluent. All major equipment used is general purpose and commercially available: the robotic arm, two automatic burettes, a vortex mixer, a centrifuge, a test tube thermostat and a personal computer. Modifications and additional small parts are easily made.

The ratio of automation to human work within the full process was a matter of optimisation considerations. These considerations may, of course, be different in different laboratories. We decided that full time attendance of one laboratory technician was affordable. His or her time could be reasonably divided between assisting the robot and doing other necessary tasks.

We had to analyse 32 to 48 samples a day. This could be conveniently done in batches of 16. The robot was working 4 h on each batch. Sample reconstitution after drying and injection into the HPLC system was done manually because the robot was too slow to handle these tasks in parallel with the sample pretreatment. Since this task required operator attendance anyway we did not take extreme measures to avoid a small amount of human intervention in the sample pretreatment. The latter was necessary because the centrifuge had to be placed on a separate table to avoid harmful vibrations. This table was difficult for the robot to reach and besides the centrifuge stopped after switch-off in a random position so that an extra correction procedure would have been necessary. Since the manual transfers of samples to the centrifuge and back took only a couple of minutes once in every 4 h, we decided that automating this step was not justified.

Thus the operator's work consisted of the following: (1) clean and dry the glassware, make and place the reagents, thaw and dispense samples, supervise the start-up of the system; (2) transfer samples to the centrifuge and back (once in 4 h); (3) reconstitute dry samples and inject into the HPLC system, and (4) control the archivation of results and register unexpected events.

This work could be done by a well-trained laboratory technician without any time pressure.

We provide here some data for evaluation of the cost efficiency of our system. We recognise, however, that such data are extremely difficult to use for comparison by a different laboratory and therefore warn from overinterpretation of the data. We needed 4 h for the automated analysis of a batch of sixteen plasma samples. This allowed analysis of 32 samples in one shift. The same number of samples could only be analysed in two shifts when the manual method was used. Moreover the automated system could be handled by a technician whereas the manual method required a full-time M.Sc. or Ph.D. analyst and a half-time technician. From these data one can estimate the labour costs per sample, but this estimate depends very much on the economic environment. Our hardware costs were estimated as US$2 per sample, excluding the costs of the HPLC equipment and any consumables. The labour saving against the manual method would return the hardware costs in appreciably less than two years (assuming 200 days of operation per year).

Compared to an automatic sample processor which can handle all liquid transfers but cannot handle

vortexing, a labour saving of 2 h 40 min is achieved per shift. If the automatic sample processor cannot transfer the organic phase after extraction to the evaporation unit (which is usually a separate device with a cover that should have to be lifted and lowered by the sample processor), this causes an appreciable delay in the process, with ensuing loss of labour efficiency. The increased need for manual assistance would very likely lead to timing conflicts, so that two technicians would have to work instead of one even if their total active working time were not fully used. Also, the increased manual intervention is likely to increase the rate of human errors.

Comparison with a fully automated robotic system is hardly possible in technical terms because with unlimited budget one can reach any technical goal. In this comparison only the lower price of the hardware can be important.

During the prolonged operation of our automatic system the following problems have been encountered. After power failure in the laboratory the system could be restarted only by manual intervention. The robot was quite sensitive to the precision of the physical dimensions of the glassware to be handled (e.g., the length of test tubes should be equal within 2 mm). Since there was no feedback from the robotic system to the computer (e.g., position sensing, liquid level sensing) the robot could not detect certain problems (e.g., if a stock solution was running out due to operator error).

The technical robustness of the system was excellent: mechanical or electric failures were very rare and there was virtually no down-time due to such problems. When the system was left standing for more than a few days we restarted it the next time one day before intended use. This was done mainly to let the HPLC system stabilise and for routine check-up.

The analytical robustness (i.e., insensitivity to environmental and operator variations) was also very good: there was no significant difference between the results of four different operators, and the system gave consistent results in a non air-conditioned room for more than a year.

5. Summary

We have shown that a multistep sample pretreatment process including liquid–liquid extraction can be conveniently and almost completely automated by a system consisting of common commercial equipment. Expensive turn-key systems are not the only possible choice for the analyst. Consideration of the particular constraints of the laboratory may allow for more economic solutions. The system shown here is fairly general: it is easily adapted for instance to other liquid–liquid extraction procedures and the commercial parts used in it can be replaced with many other similar products.

Some frequently quoted advantages of robotic systems are not fully achieved by our strategy. These would include: (1) unattended operation for 24 h and (2) no human interaction with harmful substances.

Our system requires one technician in attendance and a very limited amount of human interaction with the samples. It allows also for all important operations to be carried out in semi-darkness (light sensitive analytes).

The conclusion is that at the price of reasonable compromise robotic sample pretreatment can be extended to many areas where it has not been popular until now. This does not mean all areas, of course. The system used here would not be convenient, e.g., for the analysis of very few similar samples at a time.

Acknowledgments

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References

[1] R. Dessy, Anal. Chem., 55 (1983) 1100A.
[2] R. Dessy, Anal. Chem., 55 (1983) 1232A.
[3] R.L. Sharp, R.G. Whitfield and L.E. Fox, Anal. Chem., 60 (1988) 1056A.
[4] T.L. Isenhour, S.E. Eckert and C. Marshall, Anal. Chem., 61 (1989) 805A.
[5] H.G. Fouda, J. Chromatogr., 492 (1989) 85.
[6] K. Ozawa, P. Schnipelsky, H.L. Pardue, J. Place and A. Truchaud, J. Int. Fed. Clin. Chem., 4 (1992) 174.
[7] J. Hempenius, G. Hendriks, J. Hingstman, C.K. Mensink, J.H.G. Jonkman and C.C. Lin, J. Pharm. Biomed. Anal., 12 (1994) 1443.

[8] V.K. Boppana, K.M. Dolce, M.J. Cyronak and J.A. Ziemniak, J. Chromatogr., 487 (1989) 385.
[9] J. Guyonnet, M. Richard and P. Hellings, J. Chromatogr. B, 666 (1995) 354.
[10] S.D. Studenberg, J.D. Long, J.H. Woolf, C.J. Bruner, D. Wilson and J.L. Woolley, J. Pharm. Biomed. Anal., 13 (1995) 1383.
[11] J.H. Johnson, P. McIntyre and J. Zdunek, J. Chromatogr. A, 718 (1996) 371.
[12] I. Tsina, F. Chu, K. Hama, M. Kaloostian, Y. Ling Tam, T. Tarnowski and B. Wong, J. Chromatogr. B, 675 (1996) 119.
[13] T.L. Lloyd, S.K. Gupta, A.E. Gooding and J.R. Alianti, J. Chromatogr. B, 678 (1996) 261.
[14] R.J. Stubbs and A.J. Harker, J. Chromatogr. B, 670 (1995) 279.
[15] D.B. Whigan and A.E. Schuster, J. Chromatogr. B, 664 (1995) 357.
[16] E.C. van der Voorden, E. Gout, D.E.M.M. Vendrig and O.L.T. van Hulten, Chemometr. Intell. Lab. Syst., Lab. Inf. Manag., 17 (1992) 129.
[17] R.E. Majors, LC·GC, 9 (1995) 742.
[18] G. Horvai, A. Hrabéczy-Páll, V. Horváth and I. Klebovich, Mikrochim. Acta, 113 (1994) 171.
[19] I. Klebovich, Gy. Horvai, Gy. Grézal, K. Balogh-Nemes, V. Horváth, E. Kocsi, J. Balogh, A. Cseh and Cs. Farsang, Acta Pharm. Hung., 66 (1996) 29.