Journal of Chromatography A, 1217 (2010) 2581–2588

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Review

Monolithic methacrylate packed 96-tips for high throughput bioanalysis

Zeki Altun[a,1], Christina Skoglund[b], Mohamed Abdel-Rehim[a,c,*]

[a] Department of Chemistry & Biomedical Sciences, Karlstad University, SE-65188 Karlstad, Sweden
[b] Lantmännen Agroetanol AB, SE-60119 Norrköping, Sweden
[c] Department of Clinical Pharmacology & DMPK, AstraZeneca R&D Södertälje, SE-15185 Södertälje, Sweden

ARTICLE INFO

Article history:
Available online 31 October 2009

Keywords:
Packed 96-tips
Automated sample preparation
Methacrylate monolith
LC–MS/MS
Plasma and blood samples

ABSTRACT

In the pharmaceutical industry the growing number of samples to be analyzed requires high throughput and fully automated analytical techniques. Commonly used sample-preparation methods are solid-phase extraction (SPE), liquid–liquid extraction (LLE) and protein precipitation. In this paper we will discus a new sample-preparation technique based on SPE for high throughput drug extraction developed and used by our group. This new sample-preparation method is based on monolithic methacrylate polymer as packing sorbent for 96-tip robotic device. Using this device a 96-well plate could be handled in 2–4 min. The key aspect of the monolithic phase is that monolithic material can offer both good binding capacity and low back-pressure properties compared to e.g. silica phases. The present paper presents the successful application of monolithic 96-tips and LC–MS/MS by the sample preparation of busulphan, rescovitine, metoprolol, pindolol and local anaesthetics from human plasma samples and cyklophosphamid from mice blood samples.

Contents

1. Introduction

An ideal sample-preparation method should involve a minimum number of working steps and it should be semi or fully automated. In the pharmaceutical industry, the new strategy has led to an increase of the number of possible drug candidates. This has produced a need for high throughput in bioanalyses for toxicological and pharmacokinetic studies. Additionally, the need for same-day rotation of results from large numbers of biological samples makes high throughput bioanalysis more essential. The development of faster and higher throughput analytical methods is required for speed and capable use of time. The procedure must be highly reproducible with a high recovery of the target analytes. Because of the low concentration levels of drug in plasma and the variety of the

* Corresponding author at: Department of Clinical Pharmacology & DMPK, AstraZeneca R&D Södertälje, SE-15185 Södertalje, Sweden. Tel.: +46 8 55325604; fax: +46 8 55329026.
E-mail address: mohamed.abdel-rehim@astrazeneca.com (M. Abdel-Rehim).
[1] Present address: The Transport and Separations Group, Department of Physics, Chemistry and Biology, Linköping University, SE-58183 Linköping, Sweden.

0021-9673/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2009.10.072

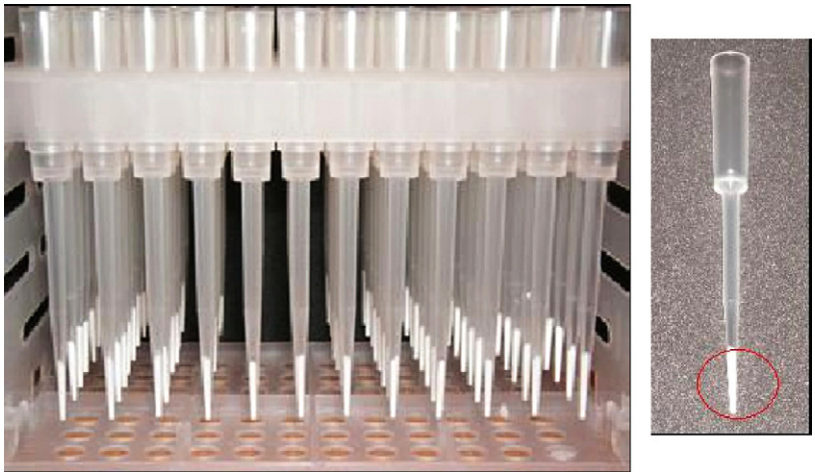

Fig. 1. UV-polymerized 96-tips packed with monolithic methacrylate polymer.

metabolites, the selected extraction technique should be virtually exhaustive.

Recent trends in the sample-preparation area focus on how to miniaturize the process, increase the sample throughput, use selective sorbents and on-line couple the sample-preparation units to separation system or detection systems [1–4].

The first attempts to miniaturize the process and provide high sample throughput were done with the introduction of new formats such as SPE disks [5], pipette tips [5–7], column switching systems [2,7–10] and multi-well plates [11]. Further, miniaturization resulted in development of new extraction techniques such as solid-phase microextraction (SPME), solid-phase dynamic extraction (SPDE), stir bar sorptive extraction (SBSE) and microextraction by packed sorbent (MEPS) [12–27]. The extraction principles SPME, SPDE and SBSE are identical and they utilize the same extraction medium, PDMS, but the amounts are different. In MEPS any sorbent material can be used either as packing bed or as coating. The sorbent material is inserted into a gas tight syringe (100–250 μL) as a plug. Sample preparation takes place on the packed bed.

In addition to these emerging new techniques, a large number of non-selective and selective sorbents has been developed to compensate for some of the drawbacks of silica-based materials, e.g. some irreversible adsorption of basic analytes.

The trend in analytical chemistry towards miniaturization of analytical systems has in sample-preparation area for instance prompted the development of new formats such as micropipette tips.

The first commercially available micropipette tip was based on chromatographic media, micro particulates C_{18}, embedded in the scaffold of a polymer (ZipTip, Millipore, Bedford, MA, USA). Since then, different types of pipette tips based on micro particulates, polymers and monoliths, and with different interaction modes such as hydrophobic, ion-exchange and affinity have been introduced [5–7,21–25]. In addition to advantages such as reduced sample- and solvent consumption, the main advantages of pipette tips based sample preparation are that it can be used with micropipettors and may be used easily with commercially available liquid-handling systems for automated high throughput applications. The advantages of organic monolithic phases over e.g. silica particles are ease of preparation, control of permeability and surface charge, no frits, and often greater pH-stability.

Recently we introduced a 96-tips set packed with a plug of a monolithic adsorbent. Using such a set it is possible to handle a 96-well plate in only 2 min [28–31,38,39]. Packed 96-tips sample preparation is a clean, high throughput, and automated sample-preparation method. Samples are prepared in a 96-well plate format and the analytes adsorb onto the polymer-based monoliths in the extraction step. The next step purifies the sample by washing the sorbent with an appropriate washing solution. In the present work, sample preparation of plasma samples was carried out, water being used as washing solution to elute salts and other polar substances. In a final step the analytes were directly eluted into a 96-well plate using an appropriate solvent for the analytes and the subsequent instrumental analysis.

2. Discussion

Solid-phase extraction is particularly attractive in drug extraction from biological samples. Highly polar compounds can be extracted from aqueous samples and then released into organic solvents on elution. For extraction of relatively polar substances using liquid–liquid extraction methods, solvent mixtures of relatively high polarity and these often yield emulsions and high matrix background retained on a reversed-phase C8 or C18 sorbents, weakly retained contaminants washed from the sorbent with aqueous buffers and finally the analytes of interest desorbed for derivatisation or direct analysis with solvents such as methanol. SPE methods are effective for complex biological samples because the main requirements of the extraction (matrix exchange, desalting, removal of macromolecules and highly polar compounds) are well matched to the properties of the sorbent. In practice, the functional failure of the silica sorbents during the extraction of biological fluids is almost always associated with blockage, coagulation of sample. In addition the deterioration caused by aggressive reagents or pH is well known. Polymeric monoliths as a SPE sorbents can give many advantages. The advantages of organic monolithic phases are such as ease of preparation, control of permeability and surface charge, no frits, and often greater pH-stability.

Organic monoliths dedicated to use in solid-phase extraction were developed and used for conventional SPE [40] and for on-chip SPE [41,42]. Our group introduced a new shape of monolithic SPE formate, this is a 96-tips set, that is packed with a plug of an organic monolithic adsorbent (Fig. 1). Our monolithic tips were prepared in two steps. First, an appropriate length of the polypropylene surface was etched and, second, the monolith, of the same length, was formed and attached to the surface. Using such a set it is possible to handle a 96-well plate in only 2 min [28–31]. Packed 96-tips sample preparation is a clean, high throughput, and automated sample-preparation method. Samples are prepared in a 96-well plate format and the analytes adsorb onto the polymer-based monoliths in the extraction step. Weakly retained contaminants were washed from the sorbent with aqueous buffers and finally

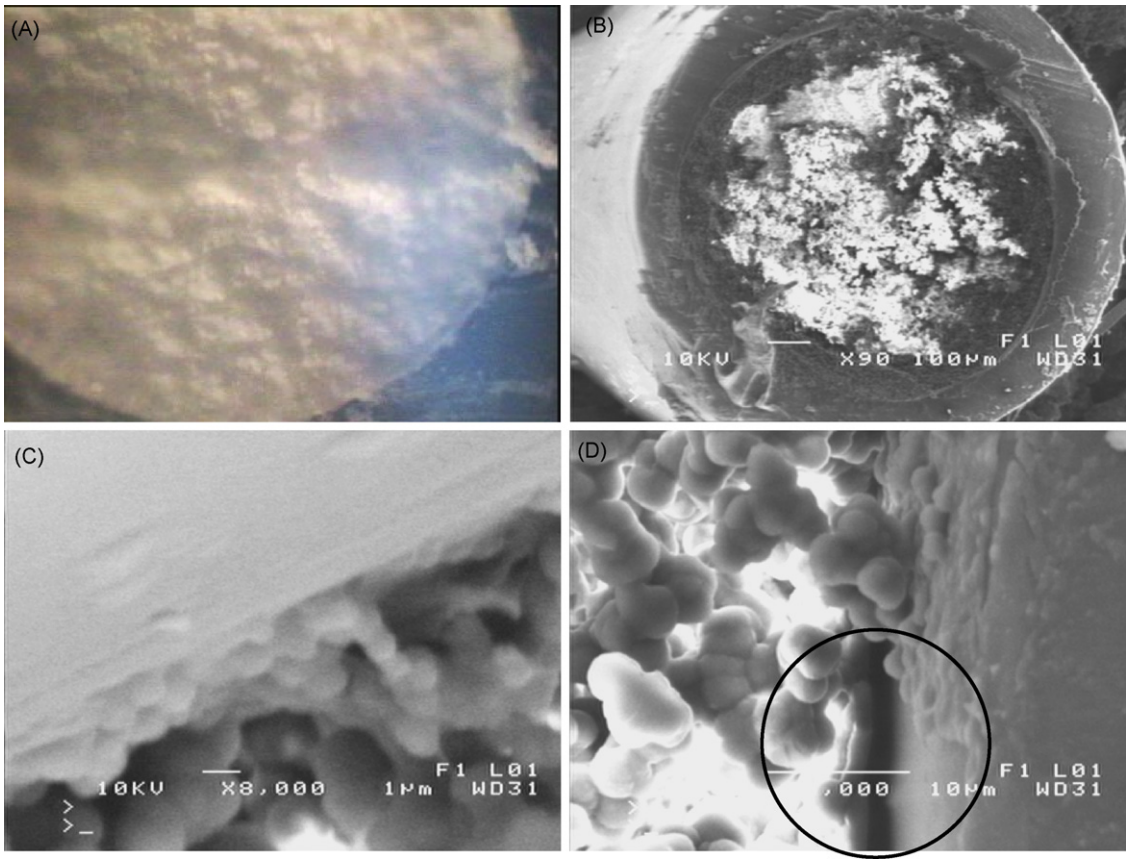

Fig. 2. (A) Photographic images of UV-polymerized monolith inside a disposable micropipette tip, Paper III. (B) SEM image of poly(ethylene glycol dimethacrylate-butyl methacrylate) monolith inside polypropylene based pipette tip, Paper IV. The tip was modified with 5 wt% BP in MeOH. No voids between plastic walls of the tip and monolith are visible. Porous monolith inside pipette tip modified with (C) MMA/EGDMA 1:1 (%, w/w) with BP (5 wt%) and (D) 5 wt% BP in methanol, The selected area indicates the gap between the plastic walls of the tip and the monolith [30].

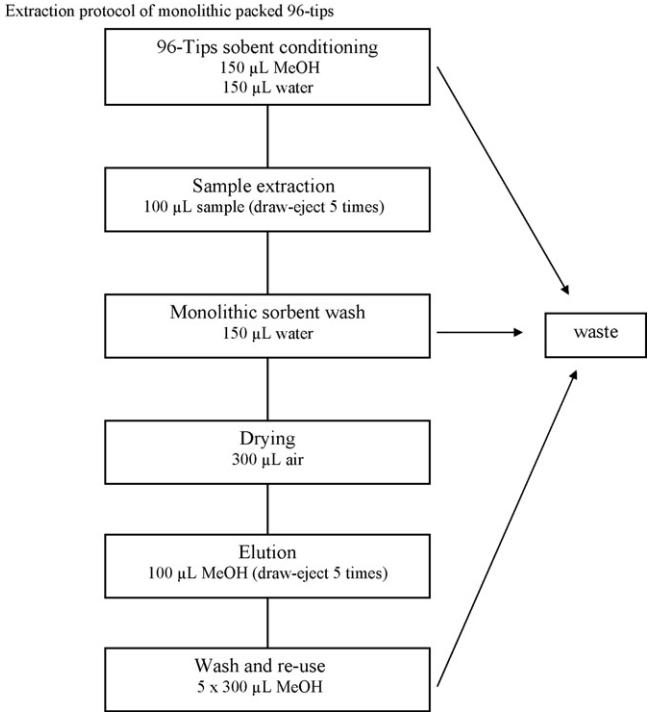

Fig. 3. Extraction protocol of monolithic methacrylate polymer packed 96-tips.

the analytes were desorbed with solvents such as methanol. For the extraction of plasma samples 96-devices have been re-used for at least 5 times. A good mechanical stability of the monolithic plug is very important to survive a complete sample-preparation run cycle (conditioning, sample loading, washing of the interferences and elution of analyte). In addition, monoliths should have good hydrodynamic properties for easy and uniform pumping (draw-eject) of sample through the bed. Thus, prior to *in situ* polymerization, the surface of the polypropylene tips should be modified [30].

3. Preparation of monolithic packed 96-tips

3.1. The preparation of monolithic sorbent at optimum conditions

The preparation of methacrylate based porous polymer monoliths and their use in chromatographic separation was published for the first time in the 1960s by Kubin et al. [32]. Since then, such monolithic supports have been used in many chromatographic areas including GC, LC, CE, CEC, SPE and microfluidic devices.

Primarily, the polymerization mixture of methacrylate based monoliths consists of the monomer(s), a cross-linker and an initiator in the presence of a combination of porogenic solvents. The preparation procedure is simple and straightforward. Basically, after mixing, the polymerization mixture is degassed using e.g. nitrogen gas in order to remove oxygen and poured into an appropriate and often surface modified chromatographic device for polymerization *in situ*, thermally or under UV light. Surface modification may be necessary in order to covalently attach the monolithic polymer to the tubing. This results in a mechanically

Table 1
Bioanalytical applications of monolithic methacrylate polymer packed 96-tips.

Compound class/compound	Sample matrix	Sample volume (μL)	Analytical method	Calibration range	Refs.
Local anaesthetics					
Lidocaine	Human plasma	100	LC–MS/MS	14–5000 nM	[28]
Ropivacaine	Human plasma	100	LC–MS/MS	2–2000 nM	[29]
Bupivacaine	Human plasma	100	LC–MS/MS	2–2000 nM	[29]
Anticancer drugs					
Cyclophosphamide	Mice blood	20	LC–MS/MS	10–2000 nM	[39]
Busulphan	Human plasma	100	LC–MS	5–2000 nM	[38]
Roscovitine	Human plasma	100	LC–MS/MS	14–5000 nM	[28]
β-Blockers					
Pindolol	Human plasma	100	LC–MS/MS	0.5–5000 nM	[31]
Metoprolol	Human plasma	100	LC–MS/MS	0.5–5000 nM	[31]

stable chromatographic device without voids forming between the monolith and the tubing walls. Before use, the monolithic material is washed with an organic solvent such as methanol to remove possible unreacted compounds.

Recently, we introduced the preparation procedure of monolithic pipette tips [28–31,38,39]. Basically, a solution containing glycidyl methacrylate (20%), ethylene glycol dimethacrylate (15.5%), butyl methacrylate (3.5%), AIBN (1 wt% with respect to monomers), 1-dodecanol (30%) and cyclohexanol (30%) was vortexed for 10 min and purged with nitrogen for 10 min in order to remove oxygen.

The pipette tips (550 μL) were filled to about 8 mm (6–7 μL) by the capillary action and placed vertically inside the polymerization apparatus, a Spectrolinker XL-1500 UV Crosslinker Spectronics Corporation (Westbury, NY, USA), calibrated at 254 nm UV light. The polymerization was allowed to proceed first for 60 min with the sharp end of the tip down and at a distance to the lamp of 15 cm, and then for 25 min with the sharp end up and at a distance to the lamp of 5 cm. After completion of polymerization the tips were removed, inspected under microscope for bubbles, and washed with acetone to remove the porogenic solvents and other compounds remaining in the monolith.

An important issue is a good mechanical stability of the monolithic plug and the monoliths should have good hydrodynamic properties for easy and uniform pumping (draw-eject) of sample through the bed. Therefore, prior to *in situ* polymerization, the surface of the polypropylene tips was modified [30]. Briefly, pipette tips were filled to about 8 mm with a methanol containing 5% benzophenone. After purging with nitrogen to remove oxygen, the tips were placed inside the polymerization apparatus (mentioned above) and irradiated using 254 nm UV light for certain time. After that, the tips were washed with methanol and dried at 40 °C for 10 min.

3.2. Factors affecting the performance of packed 96-tips

There are a number of factors affecting the porous properties of the monoliths. The factors to be considered are not only more or less all components included in the polymerization mixture such as the composition and amount of the porogenic solvents, the crosslinker, the type and amount of initiator, but also the polymerization temperature or the intensity of the UV light. Of these factors, the type and composition of the porogenic mixture seem to be the key factors most often used for fine tuning of the final properties of the polymeric monoliths. A commonly used porogenic mixture for the preparation of the monoliths is cyclohexanol and 1-dodecanol. In such a porogenic mixture the pore size seem to increase as the percentage 1-dodecanol in the polymeric mixture increases [33]. The polymerization temperature is another factor effecting pore size distribution of the monoliths. Usually, at higher temperature smaller pores are obtained. A number of papers dealing with factors effecting surface properties of monoliths have been published [34–37].

Fig. 2A–D displays scanning electron microscopy (SEM) images of the monolithic structure inside a pipette tip indicating complete filling of the polymer across the tube. No significant differences and no voids between the polymer matrix and the tip can be seen. In Fig. 2C, the SEM image of a monolith inside a pipette tip modified with MMA/EGDMA 1:1 with 5% BP shows binding between the monolith and the pipette wall. The monolith inside the tip surface modified with 5% BP in MeOH shows binding between monolith and wall in the upper part of the image (Fig. 2D). The lower part of the image shows a gap between the monolith and the pipette wall. The surface of the pipette wall in this part appears to be much smoother compared to the surface in the upper part of the image. Hence, the surface modified part seems to have obtained a more adhesive surface.

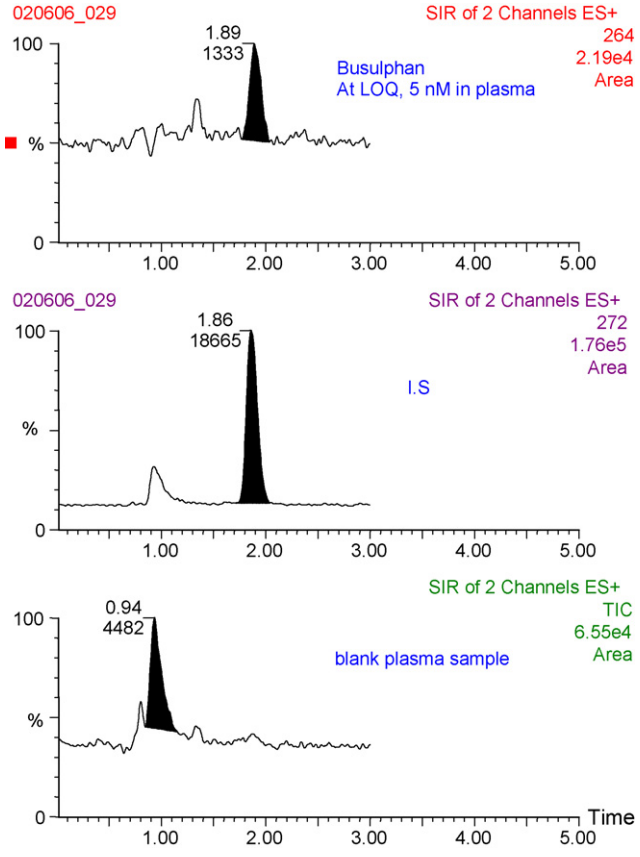

Fig. 4. Total ion chromatogram of the SIR analysis obtained from spiked and blank sample of busulphan in human plasma at LLOQ (5 nM).

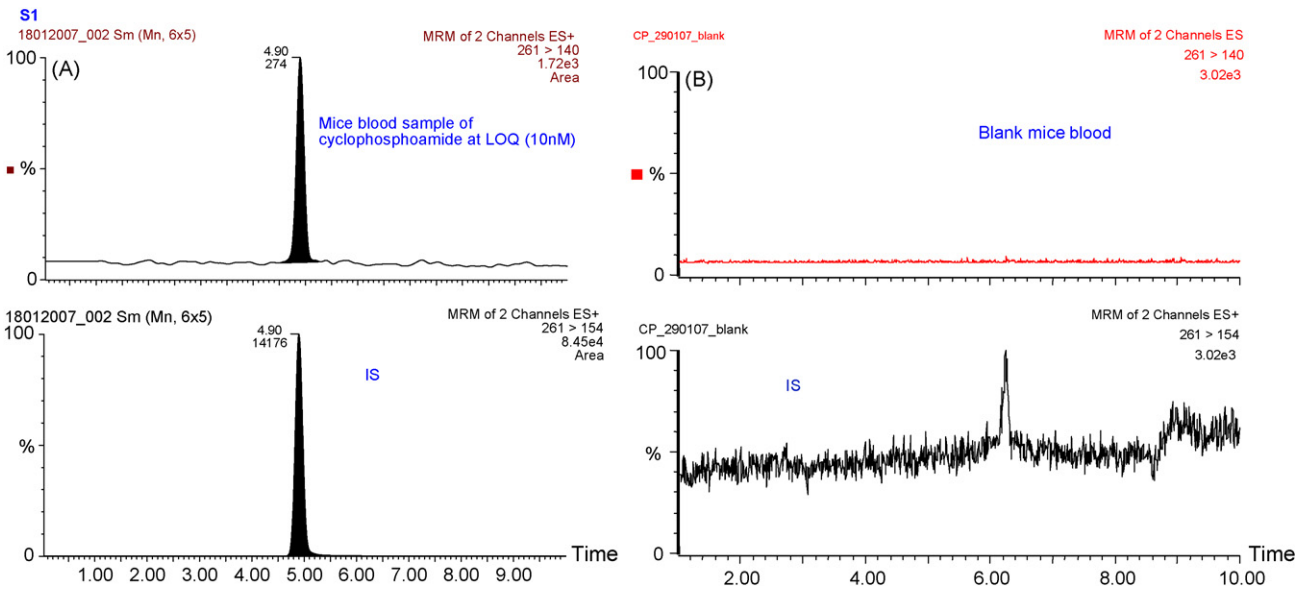

Fig. 5. Total ion chromatogram of the MRM analysis obtained from spiked and blank sample of cyclophosphamide in mice blood at LLOQ (10 nM).

3.3. Extraction procedures

Plasma samples were diluted four times while blood samples diluted 20 times with water.

- Conditioning
  The sorbent was conditioned with 150 μL methanol and subsequently with 150 μL water.
- Sample loading
  Sample can be loaded by multiple aspirates-dispenses cycles (5 × 100 μL).
- Washing step
  The sorbent was washed after sample loading by 150 μL water.
- Drying step
  The sorbent was dried with 300 μL air.
- Elution of the analytes
  Elution solution was organic solvent (≥ 60%).

In our applications the elution solution was methanol. The elution was carried out by pumping 100 μL methanol (3–5 aspirates–dispenses cycles).

In addition to robot protocol we added a pause of 20 s after sample loading, washing and elution steps to make sure all fluid had completely dripped into the 96-well plate. Also, amount of 300 μL air was aspired before each step to increase the pressure on the liquid to pass through the monolith bed. Fig. 3 shows extraction protocol for 96-tips using Personal Pipettor robot (PP-550 N-MS), obtained from Apricot Designs Inc. (CA, USA).

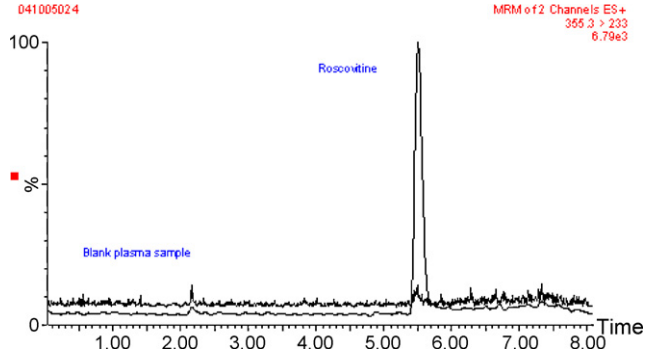

Fig. 6. Total ion chromatogram of the MRM analysis from human blank plasma and spiked plasma sample (LLOQ) with roscovitine [28].

4. Monolithic packed 96-tips applications

Monolithic packed 96-tips device was applied for extraction of drugs from biological samples. An outline for different drugs extracted by packed 96-tips is summarised in Table 1.

4.1. Anticancer drugs in plasma and blood samples

4.1.1. Busulphan in human plasma samples by LC–MS

Busulphan is an alkylating agent that is often used to treat chronic myeloid leukaemia (CML). In addition, busulphan is used in high doses as preparative regimen before stem cell transplantation (SCT). We used the packed 96-tips to extract busulphan from human plasma samples. The calibration curve in human plasma samples was in the concentration range of 5–2000 nmol/L. The validation of the method showed that the coefficients of determination (R^2) were ≥0.99 for all runs. Using six individual sources of blank matrix, the interferences from endogenous plasma compounds were less than 20% of the response of the lower limit of quantification (LLOQ). Fig. 4 shows chromatograms of busulphan, in human plasma at LLOQ and blank plasma sample. The within-day precisions (CV%) were about 4–8% (n = 6), while the between-day precisions (CV%) were in the range 7–10%. The accuracy varied from 99% to 105%. [38]. The LLOQ was set to 5 nmol/L. At this concentration the accuracy of the LLOQ was varied by ± 20% and the precision had a deviation ≤10%.

4.1.2. Cyclophosphamide in mice blood samples by LC–MS/MS

Cyclophosphamide (CPA) is one of the most widely used anticancer agents in the treatment of haematological malignancies as well as solid tumours. CPA is also used to treat some autoimmune disorders, like rheumatoid arthritis and systemic lupus erythematosus. In this work [39] the constructed calibration curves consisted of seven standard samples with a concentration between 10 and 5000 nmol/L in mice blood for cyclophosphamide. The analysis of the blank blood samples showed no significant interference peaks with the quantification of cyclophosphamide. The within-day precisions (CV%) were 4–11% (n = 6), while the between-day

precisions were in the range 15–16% ($n = 18$). The accuracy values of the QC samples were 103–108%. The lower limit of quantification (LLOQ) was set to 10 nM. At this concentration the accuracy was between 90% and 106% and the precision (CV%) was 10% ($n = 6$). Fig. 5 shows chromatograms of blank mice blood and mice blood spiked with cyclophosphamide at LLOQ.

4.1.3. Roscovitine in human plasma samples by LC–MS/MS

Monolithic packed 96-tips device was also used to extract roscovitine in human plasma samples. Roscovitine, (2-(R)-(1-ethyl-2-hydroxyethylamino)-6-benzylamino-9 isopropylpurine), has been recently considered as a possible new chemopreventive and chemotherapeutic agent [43]. The calibration curve is constructed in human plasma samples in the concentration range 14–5600 nmol/L. The "R^{2}" values for calibration curves were ≥0.999 for all runs of roscovitine. The intra-assay precisions at three different concentrations for QC samples were 5–8% while the inter-asssay precision ranged from 5% to 11%. The accuracy of QC samples varied from 95% to 104% [28]. The LLOQ was set to 14 nM and the accuracy of LLOQ was 90%. Fig. 6 shows chromatograms of blank plasma and spiked plasma samples with roscovitine at LLOQ.

4.2. β-Blocker drugs metoprolol and pindolol in human plasma by LC–MS/MS

The extraction of metoprolol and pindolol from human plasma samples was carried out using packed 96-tips in a concentration range of 5–5000 nmol/L [31,39]. The accuracy for quality control (QC) samples varied from 94% to 114% pindolol, and from 101% to 103% for metoprolol ($n = 18$). The between-day precisions (RSD) were 9–13% for pindolol, and 9–15% for metoprolol ($n = 18$). The LLOQ for the analytes studied was set to 5 nM for both analytes

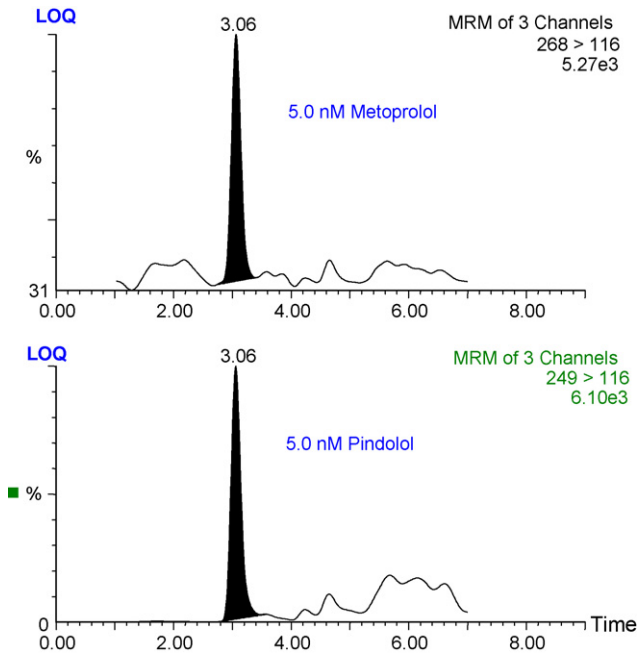

Fig. 7. Total ion chromatogram of the MRM analysis from human spiked plasma sample at LLOQ (5 nM) with pindolol and metoprolol [31].

(Fig. 7). At this concentration the accuracy of the LLOQ was between 94% and 108% and the precision had a deviation <12% ($n = 6$) for both studied analytes. Effect of elution solutions containing different amounts of methanol in water (10%, 30%, 60% and 100% methanol) on analyte response was also investigated. Highest response was obtained with 100% methanol as elution solution while it

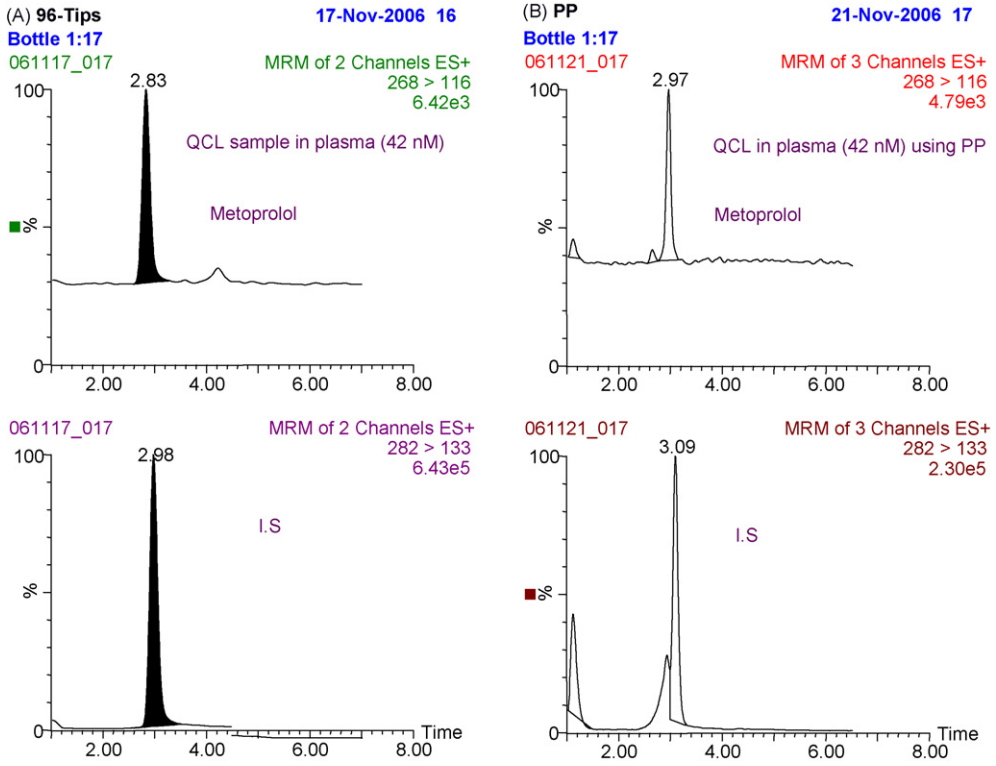

Fig. 8. Total ion chromatogram of the MRM analysis of metoprolol and internal standard from spiked human plasma sample (42 nmol/L): (A) treated by 96-tips, (B) protein precipitation was used.

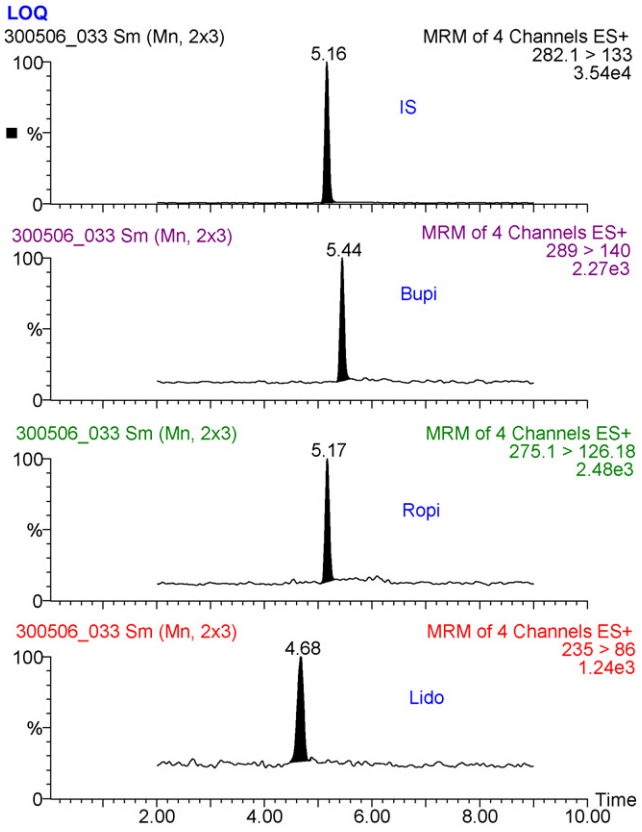

Fig. 9. Representative mass chromatograms of lidocaine, ropivacaine and bupivacaine at 2 nmol/L.

decreased by 13% when 60% methanol in water was used as elution solution.

In Fig. 8, packed 96-tips extraction method was compared with protein precipitation for the sample preparation of metoprolol and pindolol from human plasma samples. Using monolithic sorbents to clean-up the analytes, no ion suppression was detected. On the other hand, high ion suppression was observed when using the protein precipitation method (acetonitrile:plasma, 1:1 containing 0.1% HCOOH). The protein precipitation gave higher variations at all QC samples compared to 96-tips. The signal-to-noise (S/N) ratio was improved about 2-fold using packed 96-tips in comparison with PP as sample-preparation method (Fig. 8). In addition, monolithic methacrylate 96-tips were compared with commercial silica-based monolithic packed tips (OMIXC18 obtained fromVarian). The precision was within the acceptable range for the two methods, but better accuracy was obtained with methacrylate monolithic tips compared to the commercial tips [39].

4.3. Local anaesthetics in human plasma by LC–MS/MS

The use of Packed 96-tips for the extraction of the amide-type local anaesthetics lidocaine, ropivacaine and bupivacaine from human plasma samples was reported [28,31]. Fig. 9 shows chromatogram at concentration 2 nmol/L of lidocaine, ropivacaine and bupivacaine in human plasma utilizing 96-tips followed by LC–MS/MS. LC–MS/MS analysis of the blank plasma samples showed no significant interference peaks with the quantitation of ropivacaine and bupivacaine. Using six individual sources of blank matrix, the interferences from endogenous plasma compounds were less than 20% of the response of the lower limit of quantitation (LLOQ). The calibration range 2–2000 nM and the extraction recovery was 60%. The results showed good correlation coefficients ($r^2 > 0.99$) for all runs. The within-day precisions were about 3–14% for studied analytes in plasma samples, while the between-day precisions were in the range 3–17%. The accuracy varied from 101% to 118% [28,31].

5. Conclusions

A new method using monolithic methacrylate polymer bed in 96-tips was developped and recently introduced for bioanalysis application. UV-polymerization was performed *in situ* within polypropylene pipette tip after surface modification. Using a monolithic methacrylate packed 96-tips, 96 samples could be treated in 2–4 min. A good accuracy and precision could be reached. Furthermore, the present method reduces the sample-preparation time, which is of great importance in bioanalysis.

Evaluation of monolithic packed 96-tips for the extraction of drugs such as busulphan and roscovitine from human plasma and cyclophsphamide from human blood samples has been developed and validated. Whole blood samples can be handled by 96-tips and both commercial and packed 96-tips gave good accuracy and precision. Utilizing plasma samples the tips could be used several times (5 times). Utilizing blood samples, packed tips could only be used once. The results showed that the method is selective and accurate. It was shown that small sample volumes can be handled, solvent consumption was low and the procedure was fast.

References

[1] W.M. Mullet, J. Biochem. Biophys. Methods 70 (2007) 263.
[2] H. Kataoka, Curr. Pharm. Anal. 1 (2005) 65.
[3] J. Henion, E. Brewer, G. Rule, Anal. Chem. 70 (1998) 650A.
[4] E.M. Thurman, K. Snavelly, Trends Anal. Chem. 19 (2000) 18.
[5] M.W.J. van Hout, R.A. de Zeeuw, G.J. de Jong, J. Chromatogr. A 858 (1999) 117.
[6] Z. Altun, PhD Thesis, Karlstad University, Karlstad, Sweden, 2008.
[7] C.J. Kitchen, D.G. Musson, A.L. Fisher, J. Chromatogr. B 799 (2004) 9.
[8] K. Heinig, F. Bucheli, J. Chromatogr. B 795 (2003) 337.
[9] A. Holm, P. Molander, E. Lundanes, S. Øvrebø, T. Greibrokk, J. Chromatogr. B 794 (2003) 178.
[10] S. Souverain, S. Rudaz, J.-L. Veuthey, J. Chromatogr. B 801 (2004) 141.
[11] D.A. Dunn, I. Feygin, Drug Discov. Today 5 (2000) 84.
[12] R. Eisert, J. Pawliszyn, Anal. Chem. 69 (1997) 3140.
[13] R.G. Belardi, J. Pawliszyn, Water Pollut. Res. J. Can. 24 (1989) 179.
[14] C.L. Arthur, J. Pawliszyn, Anal. Chem. 62 (1990) 2145.
[15] J. Lipinski, Fresenius J. Anal. Chem. 367 (2000) 445.
[16] J. Lipinski, Fresenius J. Anal. Chem. 369 (2001) 57.
[17] J. Lipinski, Patent Application PCT/DE00/01376, 2000.
[18] E. Baltussen, P. Sandra, F. David, C. Cramers, J. Microcol. Sep. 11 (1999) 737.
[19] F. David, B. Tienpont, P. Sandra, LCGC 21 (2) (2003) 108.
[20] F. David, P. Sandra, J. Chromatogr. A 1152 (2007) 54.
[21] H.-C. Hsieh, C. Sheu, F.-K. Shi, D.-T. Li, J. Chromatogr. A 1165 (2007) 128.
[22] J.-L. Hsu, M.-K. Chou, S.-S. Liang, S.-Y. Huang, C.-J. Wu, F.-K. Shi, S.-H. Chen, Electrophoresis 25 (2004) 3840.
[23] S. Miyazaki, K. Morisato, N. Ishizuka, H. Minakuchi, Y. Shintani, M. Furono, K. Nakanishi, J. Chromatogr. A 1043 (2004) 19.
[24] S. Ota, S. Miyazaki, H. Matsuoka, K. Morisato, Y. Shintani, K. Nakanishi, J. Biochem. Methods 70 (2007) 57.
[25] A. Shukla, R.E. Majors, LCGC Eur. 1 (December) (2005).
[26] M. Abdel-Rehim, J. Chromatogr. B. 801 (2) (2004) 317.
[27] Z. Altun, L. Blomberg, E. Jagerdeo, M. Abdel-Rehim, J. Liq. Chromatogr. Relat. Technol. 29 (2006) 829.
[28] Z. Altun, L.G. Blomberg, M. Abdel-Rehim, J. Liq. Chromatogr. Relat. Technol. 29 (10) (2006) 1477.
[29] Z. Altun, A. Hjelmström, L.G. Blomberg, M. Abdel-Rehim, J. Liq. Chromatogr. Rel. Technol. 31 (2008) 743.
[30] Z. Altun, A. Hjelmström, M. Abdel-Rehim, L.G. Blomberg, J. Sep. Sci. 30 (2007) 1964.
[31] M. Abdel-Rehim, C. Persson, Z. Altun, L. Blomberg, J. Chromatogr. A 1196–1197 (2008) 23.
[32] M. Kubin, P. Spacek, R. Chromesec, Coll. Czechosl. Chem. Commun. 32 (1967) 3881.
[33] J. Hradil, F. Svec, A.A. Aratskova, L.D. Beljakova, V.I. Orlov, Y.I. Yashin, J. Chromatogr. 475 (1998) 209.
[34] F. Svec, J. Hradil, J. Coupek, J. Kalal, Angew. Macromol. Chem. 48 (1) (1975) 135.
[35] F. Svec, J.M.J. Fréchet, Chem. Mater. 7 (4) (1995) 707.
[36] D. Horak, Z. Pelzbauer, F. Svec, J. Kalal, J. Appl. Polym. Sci. 26 (1981) 3205.

[37] S. Eeltink, G.P. Rozing, W.Th. Kok, Electrophoresis 24 (2003) 3935.
[38] A. Hjelmström, Diploma Work, Karlstad University, Karlstad, Sweden, 2006.
[39] C. Skoglund, Diploma work, Karlstad University, Karlstad, Sweden, 2007.
[40] S. Xie, F. Svec, J.M.J. Fréchet, Chem. Mater. 10 (1998) 4072.
[41] C. Yu, M.H. Davey, F. Svec, J.M.J. Fréchet, Anal. Chem. 73 (2001) 5088.
[42] A. Tan, S. Benetton, J.D. Henion, Anal. Chem. 75 (2003) 5504.
[43] M. Vita, M. Abdel-Rehim, S. Olofsson, Z. Hassan, L. Meurling, A. Siden, M. Siden, T. Pettersson, M. Hassan, Eur. J. Pharm. Sci. 25 (2005) 91.