Clinica Chimica Acta 488 (2019) 196–201

Contents lists available at ScienceDirect

Clinica Chimica Acta

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

The right blood collection tube for therapeutic drug monitoring and toxicology screening procedures: Standard tubes, gel or mechanical separator?

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ARTICLE INFO

Keywords:
Separator gel
Blood collection tubes
Plasma sampling
Drug stability
LC-MS/MS
Therapeutic drug monitoring
Toxicology

ABSTRACT

Stability data of toxics or drugs in gel-based or mechanical separation blood collection tubes are lacking, especially for therapeutic drug monitoring and clinical toxicology procedures. According to ISO 15189 accreditation standard, laboratories need to master the entire preanalytical process including the stability of analytes in a specific tube. Here we explored the impact of BD PST™ II and Barricor™ separator tubes on the stability of 167 therapeutic compounds and common drugs of abuse in plasma samples using LC-MS/MS. Forty drugs were significantly affected by the use of PST™ II tubes, including antidepressants (11/26), neuroleptics (9/13), cardiovascular drugs (5/26), anxiolytics and hypnotics (4/25) and some drugs of abuse (5/26). Six compounds exhibited significant reduction by the mechanical Barricor™ tubes. Ten drugs exhibited low (< 85%) but non-significant recoveries due to inter-assay variability. Besides, a logP > 3.3 was determined as a cut-off value to predict a potential lack of stability in PST™ II gel tubes with an 86.4% sensitivity and a 61.4% specificity. As a consequence, determination of drugs with a logP > 3.3 should be carried out with caution in plasma samples withdrawn on PST™ II. The study showed the Barricor™ and non-gel tubes cause less drug interference and are recommended for the drugs studied.

1. Introduction

Separator gel barrier has been a major improvement in collection tubes for several decades. Becton Dickinson (BD) first introduced those 40 years ago and they are now routinely used in clinical laboratories. The polymer-based separator gel inside the tubes allows rapid separation of red blood cells from plasma or serum and have multiple advantages: it partially minimizes hemolysis, improves handling/storage and allows the use of primary tubes on automated analyzers, reducing needs of aliquots and minimizing pre-analytical errors [1]. In 2016, BD released a new heparin collection tube with a novel technology of separation, qualified as a mechanical separator, called BD Vacutainer® Barricor™. Instead of a gel, it uses a high-density plastic connected on elastomer top that stretches during centrifugation, finally creating a seal. Blood cells are allowed to flow around this separator reducing the time of centrifugation and providing a high-quality plasma [2]. Stability of biological parameters on separator gel tubes is a concern. Although the stability of a number of common analytes was previously assessed on automated analyzers, the stability of compounds frequently determined in plasma by high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) from gel barrier or mechanical separators remains uncertain [3–5]. Gel is highly hydrophobic, meaning that interactions with hydrophilic biochemistry parameters such as electrolytes, peptides or proteins, may be minimized [6]. However, therapeutic drugs are more likely to be hydrophobic and may be impacted by interaction with the gel. Steuer et al. studied the impact of gel separator tubes on 15 common therapeutic drugs, using LC-MS/MS, and found a correlation between physico-chemical properties and stability suggesting that lithium heparin blood collection tubes should be the preferred samples for therapeutic drug monitoring [7,8]. To our knowledge data are lacking towards the countless drugs available on the market as well as most of the drugs of abuse [9]. LC-MS/MS has become a key instrument allowing quantitation of multiple drugs concentrations at a ng/mL range with less interferences [10]. The aim of

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https://doi.org/10.1016/j.cca.2018.10.043
Received 17 August 2018; Received in revised form 31 October 2018; Accepted 31 October 2018
Available online 12 November 2018

this study was to explore the impact of BD Vacutainer® PST™ II and Barricor™ separators on the stability of a panel of 167 therapeutic drugs and common drugs of abuse (DOA) using LC-MS/MS.

2. Materials and methods

2.1. Chemicals and reagents

Drugs reference material was purchased either from Alsachim (Illkirch Graffenstaden, France), Sigma Aldrich (Saint-Quentin Fallavier, France) or LGC Standards (Molsheim, France). Of the 167 compounds that were analyzed in this study, 13 were analgesics and anesthetics, 26 antidepressants, 12 anti-infectious agents, 25 anxiolytics and hypnotics, 29 cardiovascular drugs, 26 drugs of abuse, 13 neuroleptics and 23 other drugs. LogP is a partition coefficient used to describe hydrophobicity of the chemical compounds and is a log ratio of concentrations between two liquid phases of octanol and water. A logP < 1 means a compound is more soluble in water (*i.e.* hydrophilic and more concentrated in water) and a logP > 1 is a characteristic of lipophilic compounds [11].

2.2. Blood collection tubes and sample preparation

BD Vacutainer® lithium heparin (LH) 4 mL (ref: 368884), Vacutainer PST™ II 4 mL (ref: 367374) and Vacutainer® Barricor™ 4 mL (ref: 365031) were obtained from Becton-Dickinson (Plymouth, United Kingdom). In a 50 mL flask, 10 µL of a 100 µg/mL solution containing the 167 compounds was added and evaporated to dryness under nitrogen flow at room temperature (Fig. 1). Blood from a healthy volunteer, without any medication, was drawn in BD Vacutainer® SafetyLok™ in standard lithium heparin tubes by venipuncture. Fifty milliliters of heparin anticoagulated blood were transferred to the flask and gently mixed. The final concentrations were 20 ng/mL for each compound. Two milliliters of spiked blood were manually pipetted and dropped in each of the following sets: LH, PST™ II or Barricor™. To mimic the common pre-analytical conditions of a patient sample, from sampling to analytical procedure, the tubes were stored for 4 h at room temperature, corresponding to the maximum time period allowed in our institution to accept samples for drugs analysis. Tubes were then centrifuged at 1700 *g* for 10 min at 5 °C (Thermo Scientific®, Megafuge 16R, La Jolla, CA), according to the guidelines of the French Society of Clinical Biology (SFBC) [12]. A 100 µL plasma aliquot was dropped in a 1.5 mL polypropylene tube and stored at −80 °C before analysis. All plasma samples were then thawed at the same time for LC-MS/MS analysis.

2.3. LC-MS/MS parameters

For each compound, plasma peak area was determined using a two-dimensional high-performance liquid chromatography-tandem mass spectrometry detection method (2D LC-MS/MS) developed in our laboratory. The LC-MS/MS system consisted in a quaternary and a binary pump (LC-20AD/AB, Shimadzu®, Marne-la-Vallée), an auto sampler (SIL-20 AC, Shimadzu®), a column oven maintained at 40 °C (Shimadzu® CTO-20 AC) and a 4500QTRAP tandem mass spectrometer (Sciex®, Les Ulis, France) equipped with a Turbo Ion Spray® source. Protein precipitation was performed by mixing 100 µL of plasma with 200 µL of methanol. After centrifugation at 16000 *g* for 5 min at room temperature (Centrifuge 5415D, Eppendorf®, Montesson, France), 50 µL of the deproteinized supernatant was directly analyzed into the chromatographic system. The LC-integrated online sample clean-up was performed using a perfusion column (Poros R2/20, 2.1 × 30 mm, Applied Biosystems®) and a loading phase composed of 2 mM ammonium formate in water. Chromatographic separation was achieved on an EC 50/2 Nucleodur Sphinx RP (3 µm × 50 × 2 mm, Macherey Nagel®, Hoerdt, France) and the mobile phases consisted of ammonium formate 2 mM buffer in water (A) and 0.2% formic acid in methanol (B) at flow rate of 0.4 mL/min ramped from 20% B to 80% B over 7 min. Detection and quantification were performed in multiple reaction monitoring mode (MRM) using the protonated $[M + H]^+$ precursor ions. Samples were injected following a random sequence in the LC-MS/MS system to avoid any carryover bias. The analytical procedure used

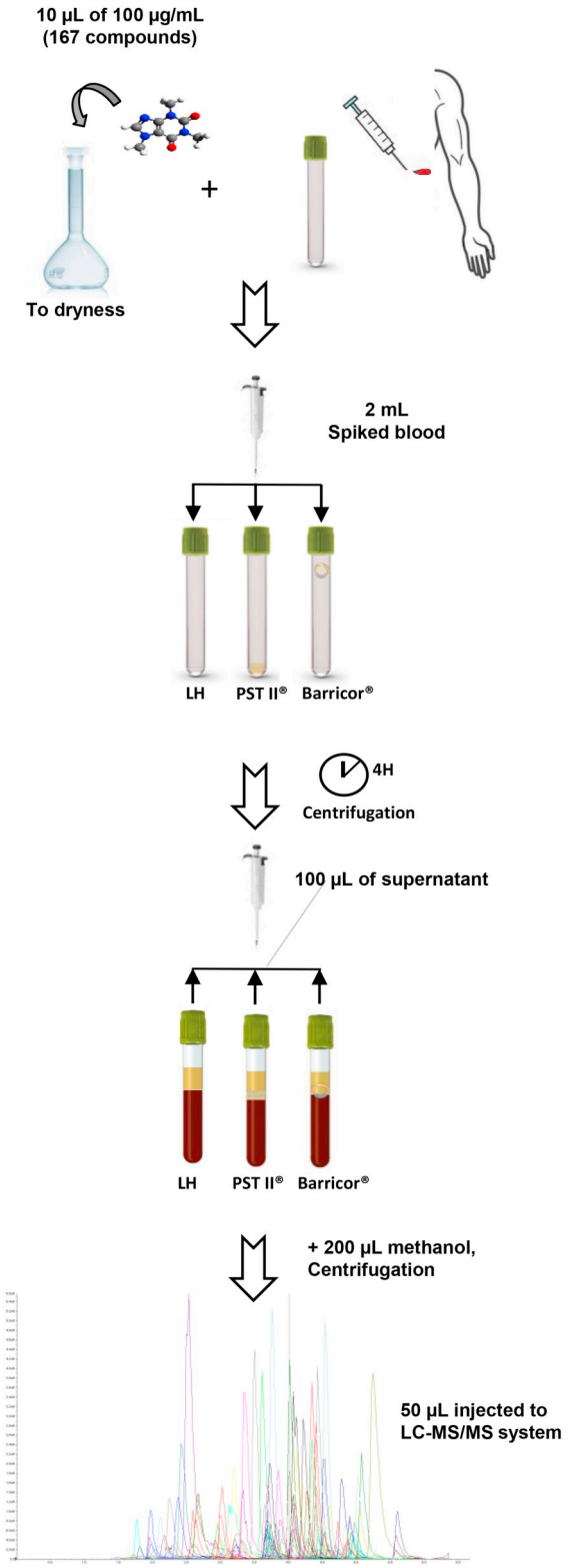

Fig. 1. * Global workflow of the experiment. *: color should be used for any print of this figure.

in this study met international analytical requirements according to ISO 15189.

2.4. Statistical analysis

The set of LH tubes without separator gel was used as a control and was considered as the reference for comparison *versus* PST™ II or Barricor™. The use of 6 independent samples for each condition allowed robust measurements and evaluation of dispersion [13]. Individual *t*-tests were used to compare mean recovery of each compound between the three pre-analytical conditions and a Bonferroni-Sidak adjusted p-value was used to avoid false discovery according to the number of parameters. A difference in mean recovery with a p-value $< 6.4E-5$ (FDR 1%) was considered as statistically significant. Statistical analyses and figures were performed using GraphPad Prism® software v7 [14].

3. Results

3.1. Impact of the PST™ II gel tubes on drugs stability

LC-MS/MS signal recoveries for each of the 167 compounds are described in Table 1. A statistical difference was observed for 40 drugs with PST™ II tubes compared to standard LH tubes (24.0%, $p < 6.4E-5$). As expected, the impact of PST™ II gel on drugs stability was variable according to the drugs families: of the 13 analgesics and anesthetics studied, 4 appeared to be significantly impacted by the PST™ II gel (30.8%) antidepressants 11/26 (42.3%), none of the studied anti-infectious agents, anxiolytics and hypnotics 4/25 (16%), cardiovascular drugs 4/29 (13.8%), drugs of abuse 5/26 (19.2%), neuroleptics 9/13 (69.2%), and other drugs 2/23 (8.7%). Interestingly, among the drugs with a lack of stability in PST™ II gel tubes, the most affected drugs were (i) some neuroleptics (including alimemazine, clozapine and its active metabolite norclozapine, cyamemazine, fluphenazine, haloperidol, levomepromazine, loxapine and promethazine) and (ii) antidepressants, especially but not exclusively tricyclic antidepressants (including amitriptyline and its active metabolite nortriptyline, buspirone, citalopram, dosulepine, doxepine, imipramine, mianserine, mirtazapine, paroxetine, sertraline, and trimipramine). These results suggest a possible role of the drug structure and/or chemical properties and led us to study the correlation between analyte stability and the hydrophilic-lipophilic balance (assessed by the logP value, Fig. 2). We observed a significant correlation between recoveries and logP value ($R^2 = 0.205$; p-value $< .0001$, Fig. 2A). A logP > 3.3 was estimated as a cut-off value at the highest likelihood ratio to predict a potent lack of drug stability in PST™ II gel tubes with a 86.4% sensitivity and a 61.4% specificity (ROC curve AUC = 0.80, Fig. 3).

3.2. Impact of the Barricor™ device on drugs stability

LC-MS/MS signal recoveries were higher than 85.5% for all drugs in the Barricor™ tubes when compared to standard LH tubes (Table 1). Six compounds were statistically impacted in Barricor™ tubes (3.6%, $p < 6.4E-5$). Among the 6 impacted compounds, we identified two antidepressants (imipramine, trimipramine), one analgesic (sufentanil), two neuroleptics (alimemazine and promethazine) and one anxiolytic (clobazam). Although only few compounds were impacted on Barricor tubes we observed a significant correlation between recoveries and logP values ($R^2 = 0.23$; p-value $< .0001$, Fig. 2B). Despite a significant difference in recoveries, the observed mean recoveries for these 6 impacted compounds were higher than 85%. According to EMA and FDA guidelines on bioanalytical method validation, a recovery within 15% of the nominal value is acceptable for LC-MS/MS drugs measurements in biological samples [15,16]. As a consequence, such variations in drugs recoveries associated to a moderate lack of stability for these compounds may be considered as not clinically relevant, according to the clinical context, by laboratories using the Barricor™ technology.

Table 1
Impact of PST™ II gel and Barricor™ system on the stability of 167 drugs, including drugs of abuse, antidepressants, anxiolytics, hypnotics, cardiovascular drugs, neuroleptics, anti-infectious agents and other drugs. Standard LH tubes were used as reference. Samples (n = 6 for each condition) were incubated for 4 h at room temperature before LC-MS/MS analysis. For each compound, plasma peak area was determined to evaluate the recovery. (*: p-value $< 6.4E-5$).

Compound	PST II Recovery (%) ± SD (%)				Barricor Recovery (%) ± SD (%)				log P
Analgesic and Anesthetic									
Codeine	92.2	±	6.7		98.9	±	9.3		1.2
Ethylmorphine	87.5	±	8.2		100.0	±	5.1		1.7
Fentanyl	80.8	±	4.3	*	94.7	±	1.9		4.1
Ketamine	84.4	±	3.5		87.9	±	4.5		2.7
Lidocaine	98.3	±	2.0		98.4	±	3.3		1.8
Morphine	88.6	±	6.8		98.8	±	4.4		1.0
N-desmethyltramadol	91.7	±	3.3		99.3	±	2.7		2.6
Nefopam	85.0	±	2.7	*	96.3	±	3.3		2.9
Norfentanyl	90.5	±	3.7		101.3	±	5.0		2.0
Norketamine	89.7	±	3.3	*	101.8	±	4.8		1.4
Oxycodone	90.1	±	4.2		103.1	±	4.6		1.0
Paracetamol[a]	93.7	±	2.9		94.8	±	3.6		0.5
Sufentanil	79.6	±	3.5	*	88.9	±	2.8	*	4.0
Antidepressants									
Amitriptyline	77.4	±	4.3	*	90.3	±	3.3		4.9
Amoxapine	86.4	±	4.7		95.7	±	4.2		2.8
Buspirone	83.6	±	2.7	*	99.5	±	6.3		2.0
Citalopram	81.3	±	6.5		94.6	±	3.2		3.5
Clomipramine	79.7	±	6.4		86.4	±	6.7		5.0
Desipramine	85.7	±	4.1		94.0	±	2.9		4.0
Dosulepine	78.7	±	3.8	*	93.4	±	4.4		5.0
Doxepine	77.0	±	5.8	*	91.3	±	4.8		4.1
Duloxetine	83.1	±	9.4		98.3	±	7.0		4.7
Fluoxetine	82.5	±	8.6		93.7	±	7.5		4.1
Fluvoxamine	87.4	±	4.9		91.8	±	2.3		2.9
Imipramine	80.8	±	2.7	*	90.5	±	3.6	*	4.8
Maprotiline	87.4	±	3.1		96.9	±	6.1		4.9
Mianserine	76.2	±	3.7	*	86.3	±	4.4		3.5
Milnacipran	94.1	±	2.5		100.7	±	4.0		1.7
Mirtazapine	82.6	±	2.8	*	95.3	±	4.7		2.9
Moclobemide	91.5	±	3.9		102.6	±	3.7		1.6
Norclomipramine[a]	84.4	±	5.2		93.7	±	6.8		3.8
Nortriptyline	84.7	±	3.5	*	94.6	±	4.6		4.7
Norvenlafaxine[a]	93.3	±	2.2		101.3	±	4.9		2.6
Paroxetine	85.6	±	2.8	*	93.9	±	4.5		3.1
Quetiapine	86.7	±	3.9		95.7	±	3.3		2.9
Sertraline	85.5	±	3.6	*	101.1	±	8.4		5.1
Tianeptine	88.9	±	3.1		96.6	±	6.6		2.1
Trimipramine	77.4	±	2.7	*	85.5	±	4.4	*	4.2
Venlafaxine	87.2	±	4.0		97.2	±	4.5		2.7
Anti-Infectious									
Amikacin	97.8	±	1.9		99.2	±	4.2		−7.4
Chloroquine	89.3	±	10.5		109.3	±	9.5		5.0
Fluconazole	95.0	±	5.1		99.9	±	3.6		0.6
Gentamicin	104.1	±	5.4		101.5	±	6.0		−3.1
Hydroxy-itraconazole	86.0	±	7.8		81.1	±	17.6		4.9
Itraconazole	89.7	±	4.3		95.8	±	8.7		5.5
Posaconazole	81.3	±	9.2		78.9	±	18.2		4.7
Quinine	83.2	±	4.0		97.2	±	3.9		2.8
Rifampicin	78.3	±	10.5		90.0	±	34.4		3.9
Tobramycin	102.7	±	8.9		97.8	±	5.7		−3.1
Vancomycin	98.9	±	5.1		97.8	±	2.8		−5.8
Voriconazole	90.8	±	3.5		94.8	±	3.5		1.7
Anxiolytics and Hypnotics									
7-Aminoclonazepam	91.9	±	2.3		101.2	±	3.1		2.7
7-Aminoflunitrazepam	88.8	±	3.8		96.5	±	4.7		1.3
Alpha-hydroxy-midazolam	86.9	±	5.0		97.7	±	3.2		3.1
Alprazolam	89.0	±	2.1	*	93.1	±	5.4		2.2
Bromazepam	93.1	±	5.0		99.3	±	4.0		2.1
Chlordiazepoxide	88.1	±	3.9		96.9	±	6.6		2.0
Clobazam	86.9	±	3.0	*	87.8	±	3.3	*	2.1
Clonazepam	82.7	±	1.9		93.4	±	2.1		2.8
Diazepam	84.5	±	4.8		85.5	±	18.8		2.6

(*continued on next page*)

Table 1 (*continued*)

Compound	PST II Recovery (%) ± SD (%)				Barricor Recovery (%) ± SD (%)				log P
Estazolam	91.4	±	3.6		96.1	±	5.5		1.7
Flunitrazepam	83.0	±	3.4		92.8	±	3.3		2.2
Hydroxyzine	82.7	±	3.2	*	99.7	±	1.8		3.4
Lorazepam	83.2	±	7.5		97.3	±	4.9		3.0
Lormetazepam	81.3	±	4.3		91.4	±	8.7		2.4
Meprobamate	94.1	±	5.7		100.6	±	2.8		1.1
Midazolam	91.0	±	3.2		97.3	±	3.6		3.9
Nitrazepam	81.2	±	4.1	*	95.0	±	2.5		2.0
Nordiazepam	84.0	±	2.7		89.6	±	9.8		2.8
Oxazepam	85.4	±	5.6		102.2	±	6.4		2.0
Prazepam	80.8	±	6.7		70.4	±	22.5		3.7
Temazepam	83.0	±	1.9		90.1	±	8.7		2.2
Tetrazepam	81.7	±	5.8		82.9	±	25.5		3.2
Triazolam	91.2	±	4.1		94.7	±	1.6		2.9
Zolpidem	87.9	±	6.7		97.8	±	0.8		3.2
Zopiclone	101.8	±	4.7		114.2	±	15.0		1.0
Cardiovascular drugs									
Acebutolol	90.2	±	2.9		100.3	±	2.2		5.2
Amiloride	92.0	±	6.3		107.0	±	5.4		−0.7
Amiodarone	97.8	±	8.6		83.8	±	26.8		7.2
Amlodipine	85.1	±	4.4		96.0	±	3.9		2.2
Atenolol	90.9	±	3.8		101.0	±	3.8		0.6
Bisoprolol	92.5	±	3.4		101.3	±	3.3		2.3
Bumetanide	82.0	±	4.7		92.9	±	7.5		3.4
Carbutamide	91.3	±	11.9		96.7	±	11.7		1.0
Carvedilol	82.9	±	3.3		97.0	±	2.2		3.1
Clonidine	86.4	±	3.8		94.8	±	3.1		2.6
Clopamide	95.1	±	4.3		100.0	±	4.3		1.9
Digoxin	97.1	±	4.5		101.5	±	5.8		1.3
Diltiazem	90.9	±	3.9		99.5	±	4.0		3.1
Disopyramide	89.9	±	3.5		100.8	±	1.8		3.2
Flecainide	85.3	±	4.4	*	97.1	±	4.1		3.0
Indapamide	88.8	±	4.6		92.3	±	1.3		2.2
Labetolol	86.5	±	4.8		100.2	±	4.3		3.1
Metoprolol	90.4	±	5.4		99.0	±	5.7		1.8
Nadolol	95.1	±	3.0		99.7	±	3.4		1.2
Nebivolol	86.8	±	5.3		94.6	±	3.6		2.4
Nicardipine	82.6	±	5.6	*	100.3	±	4.0		4.3
Nifedipine	82.9	±	6.2		86.8	±	9.0		2.5
Oxprenolol	93.7	±	3.4		103.2	±	4.1		2.4
Pindolol	87.8	±	4.7		102.5	±	3.6		2.2
Propranolol	80.8	±	9.0		93.4	±	4.4		3.0
Quinidine	86.1	±	2.8		94.4	±	5.6		2.8
Sotalol	93.4	±	2.6		103.0	±	2.2		0.9
Triamterene	90.0	±	3.1	*	100.4	±	3.6		1.2
Verapamil	80.1	±	1.4	*	96.6	±	2.2		5.2
Drugs of abuse									
6-Monoacetylmorphine	90.3	±	6.4		102.6	±	6.3		1.9
Amphetamine	90.3	±	1.9		101.6	±	3.5		1.9
Benzoylecgonine	88.1	±	3.9	*	97.2	±	3.2		1.7
Buprenorphine	76.1	±	7.4	*	93.2	±	9.4		4.5
Buprenorphine-3-glucuronide	85.1	±	2.3		109.0	±	10.6		2.8
Cocaethylene	84.5	±	2.6	*	93.6	±	3.4		2.5
Cocaine	89.8	±	4.1		97.9	±	4.6		2.0
Dihydrocodeine	90.9	±	3.8		102.3	±	3.5		1.6
EDDP[a]	92.5	±	4.0		101.0	±	4.4		5.3
Levamisole	87.8	±	3.3		99.8	±	4.9		2.2
LSD[a]	94.0	±	2.2		100.2	±	2.4		3.3
MBDB[a]	89.3	±	5.1		99.2	±	4.2		2.3
MDA[a]	89.2	±	3.4		100.6	±	6.6		2.3
MDEA[a]	93.6	±	4.2		97.6	±	4.9		2.3
MDMA[a]	92.7	±	3.8		101.8	±	4.4		1.7
Mephedrone[a]	90.1	±	3.3		95.5	±	3.6		2.0
Metamphetamine	92.0	±	2.0		100.9	±	2.5		2.2
Methadone	85.7	±	3.1	*	93.5	±	2.0		4.1
Norbuprenorphine	88.6	±	8.1		127.1	±	8.8		3.2
Norbuprenorphine-3-glucuronide	89.4	±	8.7		95.1	±	6.3		1.7
Noscapine	82.9	±	2.4	*	99.5	±	4.2		2.7
Papaverine	87.6	±	8.3		101.8	±	3.1		4.2
Pholcodine	92.4	±	3.2		104.9	±	4.9		1.1
THC[a]	99.1	±	12.0		76.0	±	7.3		7.3
THC-COOH[a]	87.0	±	2.4		79.3	±	9.3		5.2
THC-OH[a]	83.1	±	5.0		72.6	±	11.2		6.1
Neuroleptics									
Alimemazine	74.3	±	4.2	*	86.4	±	3.9	*	4.7
Amisulpride	89.4	±	3.9		103.5	±	4.1		1.5
Aripiprazole	82.8	±	5.0		94.1	±	5.6		5.2
Clozapine	82.2	±	1.3	*	100.6	±	2.4		3.7
Cyamemazine	79.1	±	2.9	*	90.3	±	6.7		4.3
Desmethyl-clozapine[a]	84.8	±	1.8	*	95.6	±	2.9		2.8
Fluphenazine	76.5	±	3.6	*	90.3	±	5.4		4.4
Haloperidol	76.3	±	5.0	*	95.7	±	2.8		4.3
Levomepromazine	76.1	±	5.3	*	88.0	±	5.7		4.8
Loxapine	77.4	±	2.3	*	85.9	±	2.6		3.2
Olanzapine	84.2	±	4.4		97.4	±	1.8		3.6
Promethazine	73.8	±	5.7	*	88.4	±	2.2	*	4.8
Risperidone	85.9	±	5.2		100.4	±	4.2		3.3
Others									
Atropine	90.0	±	7.2		101.1	±	6.4		2.2
Caffeine	86.7	±	7.3		100.1	±	9.0		−0.2
Carbamazepine	88.7	±	4.3		102.4	±	2.2		2.1
Cetirizine	89.8	±	1.6		94.4	±	3.4		3.0
Colchicine	91.7	±	4.3		91.0	±	6.1		1.6
Dexamethasone	89.6	±	5.1		93.1	±	4.1		1.9
Diphenhydramine	83.5	±	3.4	*	100.5	±	2.7		3.4
Doxylamine	89.3	±	4.6		98.3	±	7.2		2.9
Glibenclamide	81.9	±	14.6		83.1	±	31.5		3.8
Gliclazide	81.5	±	3.0	*	95.2	±	6.4		1.5
Glimepiride	85.8	±	10.5		81.4	±	21.5		1.9
Glipizide	86.7	±	7.7		98.9	±	6.5		1.9
Lamotrigine	87.1	±	5.1		96.3	±	2.0		1.9
Oxcarbazepine	91.1	±	2.3		90.7	±	2.6		1.8
Phenobarbital	98.9	±	3.5		99.8	±	5.1		1.5
Phenytoin	94.2	±	1.8	*	98.3	±	2.2		2.5
Repaglinide	77.3	±	4.5		88.4	±	13.6		5.1
Ropivacaine	90.0	±	4.9		99.2	±	3.0		2.9
Salicylic acid	98.1	±	1.6		97.7	±	1.3		2.3
Theophylline	98.5		2.3		101.4		2.4		0.0
Trihexyphenidyl	87.3	±	1.9		95.4	±	6.0		4.9
Valproic acid	97.5	±	3.8		95.2	±	2.4		2.8
Warfarin	86.7	±	4.5		88.3	±	15.9		2.4

[a] Desmethyl-clozapine (Norclozapine), EDDP (2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine), LSD (Lysergic acid diethylamide), MBDB (1,3-Benzodioxolyl-*N*-methylbutanamine), MDA (2,3-Methylenedioxyamphetamine), MDEA (3,4-Methylenedioxy-*N*-ethylamphetamine), MDMA (3,4-Methylenedioxymethamphetamine), Mephedrone (4-methyl methcathinone), Norclomipramine (desmethylclomipramine), Norvenlafaxine (desvenlafaxine), Paracetamol (acetaminophen), THC (Tetrahydrocannabinol), THC-COOH (11-Nor-9-carboxy-THC), THC-OH (11-Hydroxy-Δ9-tetrahydrocannabinol).

Despite a high specificity of a logP value > 5.4, as assessed by ROC curve analysis (data not shown), to predict a potent lack of drug stability in Barricor™ tubes, sensitivity was not sufficient with a 95% confidence interval of 6.7–66.3.

4. Discussion

Data regarding the stability of exogenous compounds such as toxics or drugs in gel separator tubes and Barricor™ technology tubes are lacking, especially for therapeutic drug monitoring and clinical toxicology procedures. ISO 15189 standards can be used by clinical laboratories in developing their quality management systems and assessing their own competence. According to these accreditation standards, laboratories need to master the entire pre-analytical process including the stability of analytes in a given tube, a biological matrix and under specific storage conditions. The aim of this study was to assess a difference in the stability of each compound in the presence of the PST II™ or the Barricor™ separators compared to standard LH tubes. The

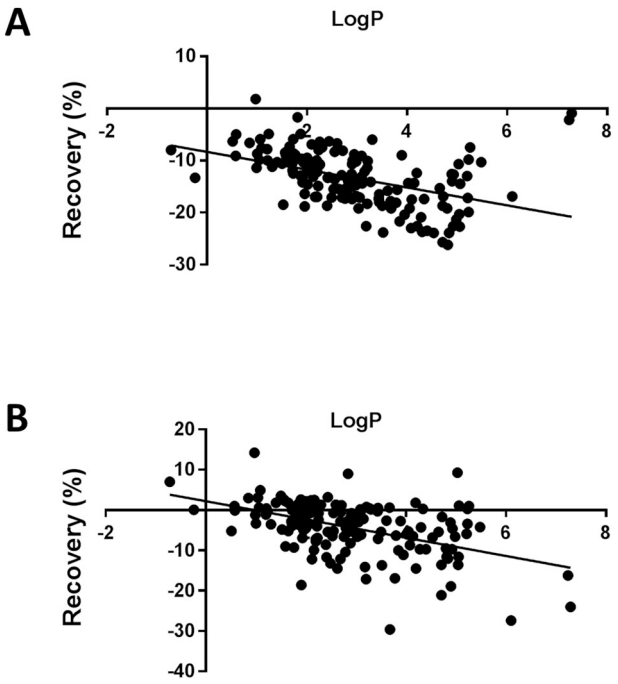

Fig. 2. (A) Difference of recovery (%) between PST™ II and standard lithium heparin tubes as a function of logP (n = 167, $p < 0.0001$, $R^2 = 0.205$) (B) Difference of recovery (%) between Barricor™ and standard lithium heparin tubes as a function of logP (n = 167, $p < 0.0001$, $R^2 = 0.23$).

presented LC-MS/MS screening procedure is a powerful tool, particularly suitable for this kind of study. The pre-analytical conditions selected for this analysis seem appropriate to the treatment conditions of samples widely accepted in the pharmacology and toxicology laboratories for most analytes (delivery times, storage conditions for biological samples). During this study the centrifugation conditions applied for each set of tubes were those recommended by the French Society of Clinical Biology standard LH tubes in order to make sure that the only difference between the tubes was the presence or not of a separator [12]. However, according to technical datasheets for Barricor™ and recent publication by Dimeski and Johnston this centrifugation setting is too low to ensure the mechanical device is properly seated between the cells and plasma and achieve complete separation [9]. Therefore, further studies may take this point into account by applying specific centrifugation settings for each set of tubes.

The observed drug recoveries in plasma samples were significantly different for a number of drugs using PST™ II gel blood collection tubes when compared to standard LH tubes. Results suggest that PST™ II blood collection tubes are suitable for the determination of most compounds in plasma but should not be recommended for the determination of some antidepressants, neuroleptics as well as other drugs that were significantly impacted by the gel as presented in Table 1. Barricor™ tubes exhibited superiority over PST™ II gel tubes, with a low proportion of impacted analytes. Although statistically relevant variations were observed using the Barricor™ tubes, slight variations of drugs recoveries may not be clinically relevant. Moreover, while 6 compounds showed a significant decreased stability in Barricor, for 10 compounds a noticeable but statistically non-significant decreased recovery (mean < 85%) was observed and does not allow us to state wether Barricor™ can impact their stability or not. These included hydroxy-itraconazole, posaconazole, prazepam, tetrazepam, amiodarone, THC, THC-COOH, THC-OH, glibenclamide and glimepiride. For these drugs, Barricor™ tubes should not be recommended unless further studies are conducted. The introduction of a criterion taking into account the clinical relevance for each compound may be useful to discriminate between a statistically significant variation and a patient management decision threshold, depending on the clinical context of the assay. Total allowable error in analytical procedure may also be used as a criterion but depends largely on the chemical parameters of the analyte.

Unlike other drugs for which a therapeutic adjustment may be subsequent to the dosage, in most cases of the tested DOA that were impacted by the PST™ II gel tubes, differences of recoveries may be less crucial. Indeed, most of the toxicological screenings are intended as qualitative tests to assess the presence or not of a drug. However, such impact can be considerable when the drug is found at concentrations close to the limit of detection and/or quantification, according to each laboratory procedure. Due to patented formulation, composition of the separator gel inside the tube is unknown but we hypothesize that the difference in drug recoveries between PST™ II and Barricor™ may depend in part on the volume/weight of gel and the contact surface with the sample that is higher in PST™ II than in Barricor™ blood collection tubes. As an explanation, the barrier gel may adsorb compounds from the plasma. To further explore this observation, we estimated the correlation between mean recovery and logP (octanol/water partition coefficient characterizing the drug hydrophilic-lipophilic balance). LogP is a physico-chemical parameter often used to describe the hydrophobicity of a chemical substance [15]. The linear regression of mean recoveries of LH against PST™ II exhibited a significant correlation (Fig. 2A) indicating that hydrophobic compounds may have a better affinity for separator gel and undergo a decrease in concentration. These data suggest that logP is an important contributor in drug stability when using PST™ II gel separator blood collection tubes. Surprisingly, some drugs with high logP values, including Δ9-Tetrahydrocannabinol (THC) and its metabolites, were not affected by the gel and point out the fact that other parameters may contribute to drugs stability when using this type of blood collection tubes. Combining data from hydrophobicity and a 15% acceptable bias according to standard validation guidelines for drugs determination using LC-MS/MS in biological samples, we proposed a logP cut-off value of 3.3 to predict the behavior of compounds that were not tested here [15,16]. At a 15% decrease in drug signal recovery, the ROC curve analysis (Fig. 3) indicated that a logP value over 3.3 was associated to a decreased drug stability. Therefore, we propose a simple mean to assess the behavior of a given compound towards the gel by using its logP. A value below 3.3 may generally allow the quantitation of a compound in blood drawn on PST™ II collection tubes. Spiked samples might provide a higher adsorption of drugs in the gel due to low plasma protein binding, and the tubes were filled with 2 mL instead of the 4 mL recommended by the

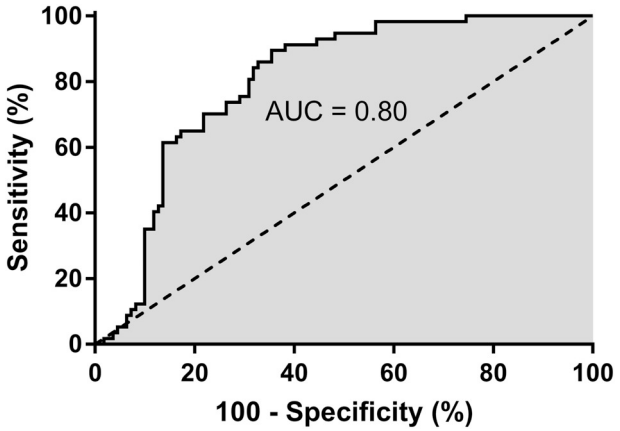

Fig. 3. ROC curve showing the predictive value of a logP > 3.3 to assess the stability of drugs in PST™ II gels barrier tubes (n = 167 compounds).

provider, therefore some compounds are likely to be not as much affected in real samples [17]. Similar to PSTII™ tubes, we observed a significant correlation between LogP and recovery in Barricor™ tubes, suggesting that the mechanical separator is not totally inert. However, due to a lack of sensitivity and despite a good specificity, a logP cut-off value of 5.4 for Barricor™ tubes does not appear good enough for routine use.

These data were obtained from spiking experiments and we cannot exclude any interference with *in vivo* drugs metabolites. However, more than twenty metabolites that usually observed *in vivo* were included in our experiments. This study was performed according to FDA and EMA guidelines [15,16] and using six technical replicates we report similar results to those previously published by Steuer et al. [7]. In addition, some drugs displaying a high logP value were not highlighted as impacted by the separator medium due to a high variability in recovery. A higher number of replicates could point out a statistical relevance for such compounds.

As a conclusion, separator blood collection tubes are associated with a decrease in many drug concentrations. The impact of gels may vary according to their composition as well as the considered drug classes and their physico-chemical characteristics. We suggest that logP is an important contributor in drug stability when using separator blood collection tubes such as PST™ II. A logP cut-off value of 3.3 may discriminate drugs with a decreased stability in these collection tubes. Determination of drugs such as antidepressants, neuroleptics and some others reported in this study should be carried out with caution when obtained from gel barrier separator. According to the observed improvement of Barricor™ over PST™ II on drugs stability, mechanical technology such as Barricor™ and/or standard blood collection tubes should be recommended for such drugs. However, for the 10 compounds exhibiting a noticeable but statistically non-significant decreased recovery with Barricor™ tubes, further studies are needed to clarify the impact of the mechanical separator on their stability.

Acknowledgments/conflicts of interest

This study was funded by the Rouen University Hospital. Collection tubes used in this study were generously provided by Becton Dickinson. The authors have no conflict of interest to disclose.

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