

1

2 **Accuracy of spinal anaesthesia drug concentrations in mixtures prepared by**
3 **anaesthetists**

4

5 Michael Heesen, Christian Steuer, Peter Wiedemeier, David Schwappach

6

7 Michael Heesen,¹ Prof. Dr., Christian Steuer,² PhD, Peter Wiedemeier,³ PhD, David Schwappach,^{4,5}
8 Prof. Dr.

9

10 ¹Department of Anaesthesiology, Kantonsspital Baden, Baden, Switzerland

11 ²Institute of Pharmaceutical Sciences, ETH Zürich, Zürich, Switzerland

12 ³Hospital Pharmacy, Kantonsspital Baden, Baden, Switzerland

13 ⁴Swiss Patient Safety Foundation, Zürich, Switzerland

14 ⁵Institute for Social and Preventive Medicine, University Bern, Switzerland

15

16

17 Correspondence:

18 Michael Heesen

19 Im Ergel 1

20 5405 Baden

21 Switzerland

22 michael.heesen@ksb.ch

23 +41-56-486-3102

24

1 **Abstract**

2 **Objective:** Medication errors include the indirect dosing of drugs. For spinal anaesthesia mixtures of
3 local anaesthetics and opioids are drawn from ampoules and combined in a syringe, according to
4 clinical practice. We set out to determine the accuracy of the drug mixtures.

5 **Methods:** Physicians of our department were invited to prepare the mixture used for spinal
6 anaesthesia for caesarean section, consisting of 10 mg hyperbaric bupivacaine (2 mL volume), 20 µg
7 fentanyl (0.4 mL volume), and 100 µg morphine (0.1 mL volume). Concentrations of these drugs were
8 determined by means of high performance liquid chromatography. Inter- and intraindividual
9 variations were assessed.

10 **Results:** We analyzed 96 samples from 31 physicians. 51% of the measured bupivacaine
11 concentrations were in +/- 10% deviation range of the intended concentration; 17% of the fentanyl
12 and 24% of the morphine concentrations were in this range. 2.1% of the samples had a bupivacaine
13 concentration corresponding to a dose ≤ 8 mg, and 11.5% of the samples had a morphine
14 concentration corresponding to a dose ≥ 150 µg. Intraindividual variations were 10.9% for
15 bupivacaine, 24.7% for fentanyl, and 38.9% for morphine.

16 **Conclusions:** Our results show a high deviation of the obtained from the intended concentrations. 2%
17 of the samples had bupivacaine concentrations that probably result into an insufficient analgesia,
18 11% of the samples had morphine concentrations that according to guidelines would require a longer
19 monitoring period than with the intended dose.

20

21

22 **Key words:** medication error, spinal anaesthesia, bupivacaine, fentanyl, morphine

23

24

25

1 **Medication errors still pose a serious threat to patients undergoing anesthesia. In clinical practice**
2 **different drugs are drawn from ampoules into syringes for further injection which may lead to**
3 **inaccuracies in volume administered. This is particularly a problem when a dilution is prepared and**
4 **when the volumes diluent and solvent may deviate¹ so that there is the risk of under- or overdosing**
5 **of the drug. The most frequently used anaesthetic technique for Caesarean section is spinal**
6 **anaesthesia^{2,3} for which a combination of local anaesthetic and opioid is frequently used.⁴ In line with**
7 **current practice⁵, in our institution we use hyperbaric bupivacaine in combination with fentanyl and**
8 **morphine opioids which provide short and long-lasting postoperative analgesia. The use of three**
9 **different drugs with different volumes multiplies the risk of under- or overdosing. This process of**
10 **drawing up and mixing different drugs relies heavily on the individual skills and performance of the**
11 **anesthesiologist. Surprisingly, little is known about the reliability of the process and the accuracy of**
12 **the prepared drug doses.**

13 We performed a study where spinal anaesthesia was prepared according to the standard protocol in
14 our operating room and then analysed the concentrations of these three drugs by liquid
15 chromatography coupled to mass spectrometry. We hypothesized that the deviations from the
16 intended concentration will be highest for the drug with the lowest volume. We present our results
17 and discuss the clinical implications of either a too high or a too low dose.

18

19

20

21

22

23

24

25

26

27

28

29

30

31

32

33

34

1 **METHODS**

2

3 **Preparation of combined drug samples**

4 Both consultants and residents were invited to participate in this study. Participation was on a
5 voluntary basis and data were blinded. Level of training was the only personal information registered
6 with samples. The participants drew up 2 mL of hyperbaric bupivacaine 0.5% (10 mg) and 0.4 mL of
7 fentanyl 50 µg /mL (20 µg) into a 5 cc syringe. Then they withdrew 0.1 mL of morphine into an insulin
8 1 ml syringe and subsequently transferred the contents from this syringe into the 5 mL syringe.
9 Unfortunately, by error ampoules with a wrong concentration of morphine were used for this study
10 (10 mg/mL instead of 1 mg/mL). Thus the final volume in the 5mL syringe was 2.5mL. Of the final
11 mixture, 1.5 mL was transferred to a 1.5 mL vial which could be deep frozen for analysis at a different
12 date. Physicians were asked to prepare several samples. Ethical approval was not required for this
13 study.

14 **Analysis of drug concentrations**

15 Nowadays, compatibility and stability studies, therapeutic drug monitoring and analytical quality
16 control of pharmaceutical drug preparations are done using high performance liquid chromatography
17 coupled to ultraviolet detectors (HPLC-UV) or mass spectrometric analysis (HPLC-MS). Identification of
18 molecules is done by chromatographic retention time comparison and/or matching fragmentation
19 pattern of reference compounds. In selected carrier solutions, target analytes are selectively
20 quantified against corresponding reference standards. Several reports are published showing the
21 beneficial and ubiquitously applicable workflow of these instrumental procedures.^{6,7,8}

22 **Chemicals and reagents**

23 Formic acid (FA), sodium chloride and analytical reference standards of morphin, fentanyl and
24 bupivacaine were obtained from Sigma Aldrich (Buchs, Switzerland). Acetonitrile Optima LC/MS
25 Grade was obtained from Thermo Fisher Scientific AG (Sunnyvale, CA, USA). Gibco™ PBS pH 7.4 (1X)
26 was purchased from Life Technologies Limited (Paisley, UK). Pure water was obtained from an in-
27 house water purification system from Labtec (Villmergen, AG, Switzerland). HPLC vials, screwcaps
28 and inlets were purchased from BGB (Boeckten, Switzerland). For all experiments, Gilson pipettes
29 and Gilson DIAMOND tips were used (Mettmenstetten, Switzerland).

30 **Chromatographic conditions**

31 All samples were analysed on a Waters Acquity UPLC system equipped with an autosampler, a binary
32 pump and a column oven (Waters, Milford (MA), USA). Detection was done on a Thermo LTQ XL
33 linear ion trap mass spectrometer (Thermo Scientific, San Jose (CA), USA). For all separations, water
34 with 0.1% FA (solvent A) and ACN with 0.1% FA (solvent B) were used. Gradient elution was done on
35 a VWR ACE Excel C8 column (2.1*30 mm, 2 µm). Chromatographic conditions were as follows: flow
36 was set 0.4 ml/min; 0 - 1 min 98% A, 1 - 7.5 min to 100% B, 7.5 - 8.5 min 100% B, 8.5 - 9,0 min to 98%
37 A, 9-10min, 98%A. Column oven temperature was set to 30 °C. Autosampler was kept at 10 °C and
38 injection volume was 3 µl. Settings of the mass spectrometry (MS) detector are used as reported
39 previously.⁸ Analysis was done in ESI positive mode. Heated ESI II source was set to 150 °C. Sheath

1 gas 40 arbitrary units (AU); auxiliary gas 20 AU; source voltage 3.00 kV; ion transfer capillary 300 °C;
2 capillary voltage 31 V; tube lens voltage 80 V. Automatic gain control was set to 15000 ions for full
3 scan and 5000 for MSn. Collision induced dissociation (CID)- MSⁿ experiments were performed on
4 precursor ions selected for MS1. Using information dependent acquisition. MS1 was performed in
5 full scan mode (m/z 100-500). MS2 and MS3 were performed in the IDA mode: four IDA MS2
6 experiments were performed on the four most intensive signal from MS1 and additionally eight MS3
7 scan filters were chosen to record the most and second most ions from MS2.

8 **Sample preparation**

9 Calibration solutions (CAL) and quality control solutions (QC) were prepared from independent stock
10 solutions in 0.9% (w/v) sodium chloride. Concentration of stock solutions were 400 µg/ml morphine,
11 40 mg/ml hyperbaric bupivacaine and 80 µg/ml fentanyl, respectively. For CAL, Stock solutions (1 mL
12 each) were mixed and filled up to 10 mL with 0.9% sodium chloride solution to obtain final
13 concentrations. QCs were prepared accordingly using 0.9 mL of stock solutions. **For analytical**
14 **standard preparation, density differences of hyperbaric bupivacaine solutions were not taken into**
15 **account.**⁹ For analysis, 25 µL CAL, QC and therapeutic solutions were diluted with 975 µL mobile
16 phase A. All samples were analyzed in independent duplicates and quantified against CAL solutions.

17 The concentration of therapeutically used morphine solution was given as 100 mg/mL of the
18 corresponding hydrochloride trihydrate salt in the commercial available finished product. According
19 to the diluting scheme, this results in a final concentration of 304 µg/mL of the free morphine base.

20 **Data analysis**

21 Descriptive statistics were used to analyze the data. We report mean, absolute and relative
22 deviations from the target concentration. Samples were also classified in terms of their relative
23 deviation from the target concentration (+/- 0-5% deviation; +/- 5-10% deviation; +/- 10-20%
24 deviation; +/- 20-40% deviation; > +/- 40% deviation).

25 We also assessed intraindividual variations by analyzing the data of physicians who prepared ≥2
26 samples.

27

1 RESULTS

2 In total, 96 samples by 31 physicians were obtained. Up to six independent preparations per
3 physician were investigated in detail.

4 Accuracy of the LC-MS procedure for morphine, bupivacaine and fentanyl was 98%, 101,5% and
5 100.5%. No matrix effects were observed for any target analyte. Overall recovery was higher than 97%.
6 Autosampler stability was given for 48 h. Multianalyte Cal and QC samples were stable for at least two
7 freeze thaw cycles.

8 Table 1 shows the deviations of observed from intended concentrations of the three drugs.

9 For bupivacaine (target concentration 4 mg/mL), the observed concentration was 3.7 mg/mL The
10 mean absolute deviation was 0.47 mg/mL. Only 49 samples (51%) had maximum deviations of +/- 10
11 % of the targeted concentrations. Two samples (2.1%) had bupivacaine concentrations \leq 3.2 mg/mL,
12 which would correspond to a dose of 8 mg in 2.5 mL final volume. This is a clinically relevant cut-off
13 for potential underdosing.

14 For Fentanyl (target concentration 8 μ g/mL), the mean observed concentration was 7.9 μ g/mL. The
15 mean absolute deviation was 2.09 μ g/mL and 16 samples (17%) had concentrations within a +/- 10%
16 deviation range of the target.

17 For morphine (target concentration 304 μ g/mL) the observed concentration was 265.6 μ g/mL. The
18 mean absolute deviation was 102.03 μ g/mL. Of the samples, 23 (24%) had deviations in morphine
19 concentrations of +/- 10% of the target. However, 11 (11.5%) samples had morphine concentrations
20 \geq 456 μ g/mL, this would correspond to a dose of \geq 150 μ g if we had not confused the 1 mg/mL
21 ampoules with 10 mg/mL ampoules in the experiment.

22 2.1% of the samples had a deviation of more than 40% of the intended concentration of bupivacaine;
23 this deviation was found for fentanyl in 17.7% and for morphine in 38.5% of the samples.

24 Figure 1 presents the distributions of observed concentrations relative to the intended
25 concentrations for all three drugs.

26 Only one sample had all drugs within a +/- 0-5% deviation range. Contrary, in 25 samples (26.0%) all
27 drugs deviated at least +/- 10 % of the targeted concentrations.

28 The intraindividual variations were 10.9% for bupivacaine, 24.7% for fentanyl, and 38.9% for
29 morphine; individual data are given in Figure 2.

30

31

1 DISCUSSION

2 In our study, observed drug concentrations prepared as in routine practice for caesarean section
3 deviated considerably from the intended concentrations. Our hypothesis that drug concentration
4 deviations would be highest for the drug with the lowest volume (morphine) was clearly confirmed.

5 The clinical relevance of our study lies in the under- or overdosing of the components of our drug
6 mixture. It has been shown in a meta-analysis¹⁰ of randomized controlled trials that the dose of
7 bupivacaine used for spinal anaesthesia in patients scheduled for caesarean section is
8 important for the anaesthetic efficacy. In their analysis of 12 RCTs Arzola and Wieczorek¹⁰ were able
9 to demonstrate that doses of ≤ 8 mg bupivacaine were associated with a significantly increased need
10 for analgesic supplementation during caesarean section compared with higher doses; the risk ratio
11 was 3.76 (95% confidence interval 2.38, 5.92). This result was confirmed even after correcting for
12 confounding variables. In our study, 2% of the samples contained a bupivacaine concentration
13 corresponding to less than 8 mg. This suggests that insufficient anaesthesia seems to a rather rare
14 occasion.

15 Conversely, we also have to discuss the consequences of a drug dose that is higher than intended.
16 This concern applies to morphine, a hydrophilic opioid that spreads cephalad in the cerebrospinal
17 fluid. Morphine can reach the respiratory centers and cause a respiratory depression that can occur
18 as long as 2 hours after the intrathecal administration.¹¹ There are reports of patients who suffered
19 from respiratory depression requiring immediate treatment.^{12,13} A systematic review of the available
20 evidence on neuraxial opioids (including intrathecal and epidural application) found an incidence of
21 clinically significant respiratory depression of 1.08 – 1.63/10,000 for clinically relevant doses of
22 opioids.¹⁴ In response, the American Society for Obstetric Anesthesia and Perinatology issued a
23 consensus statement on the monitoring of obstetric patients who received neuraxial opioids¹⁵
24 where the recommended monitoring periods depend upon the morphine dose. They suggested a
25 tiered monitoring system; for 100 μg they recommended a monitoring period of 12 hours with blood
26 pressure and sedation score examination every 2 hours whereas for doses ≥ 150 μg the monitoring
27 period should be 24 hours with hourly checks for the first 12 hours and once every two hours for the
28 next 12 hours. Our analysis showed that the morphine concentration was higher than 50% of the
29 intended dose of 100 μg in 11 of the analyzed samples. Our study therefore reveals a serious safety
30 concern with the standard procedure of preparing the spinal drug mixture in our institution. In a
31 significant percentage of cases the applied morphine dose is 150 μg morphine instead of 100 μg but
32 the monitoring period in our institution is that of 100 μg . Patients may be exposed to the risk of an
33 opioid-induced respiratory depression and this may go unnoticed. Vital consequences for the patient
34 may result as well as legal consequences for the anaesthetist. It is concerning that in more than one
35 fourth of our samples, all drugs had a deviation of more than $\pm 10\%$ of the target concentration.
36 However, from a human factor perspective, the outcome of our study is not surprising. The process
37 of drawing different drugs from ampoules into syringes seems seriously error prone in itself. In
38 particular, after the first drug has been injected into the syringe, multiple errors can potentiate and
39 there is little chance or guidance for the clinician to check the concentrations produced. As a major
40 conclusion from our study we think that the drug mixture should no longer be prepared by the
41 anaesthetist but should be provided by the pharmacy or the drug manufacturer so that the risk of
42 under- or overmedication can be avoided. **Previous study examined the stability of mixtures of**
43 **bupivacaine with one opioid in polypropylene syringes and PVC cassettes.**^{16,17} **We still need data on**
44 **the stability of the mixture of hyperbaric bupivacaine, morphine, and fentanyl in the**
45 **concentrations used in our study before ready-to-use mixtures can be recommended.**

1 Limitations: Because we present an observational study of a single group, a power calculation was
2 not possible and therefore we could not draw conclusions on statistical significance. **In our study we**
3 **erroneously used a concentration of morphine that is 10 times higher than the concentration we**
4 **use in our clinical practice; however, we do not think that this fact has an impact on our**
5 **conclusions.** The results of our study must be interpreted in clinical context. We think that our
6 finding that 11 of the 96 samples showed a morphine dose that was $\geq 50\%$ higher than what was
7 intended is an impressive number. Whereas we cannot conclusively say whether this can be
8 generalized and whether they would be found with participants from another anaesthesia
9 department, we have no reason to believe that other physicians would perform significantly better
10 than the participants in this study. A study limitation is that we did not assess association between
11 experience (trainee versus specialist, specialist with several years versus specialist with only a few
12 years of clinical experience) and accuracy of the drug preparation. We believed that our cohort was
13 too small to have a sufficiently high number of participants in the various subgroups. Another
14 complication that we did not address is bacterial contamination, a complication that can occur when
15 physicians prepare medication. Our study mixtures were prepared under the same sterile conditions
16 as during our daily clinical practice and in the last years we have not seen meningeal or
17 other infection due to contamination of the drug mixture. This finding, however, does not preclude
18 the fact that bacterial contamination can occur and may cause serious sequelae in patients at an
19 increased risk of infection, eg immunocompromised patients.

20

1 **CONCLUSIONS**

2 We found a high percentage of samples that a concentration different from the intended
3 concentration. The difference was the highest for the drug with the smallest volume. There was also
4 considerable intraindividual variation. As a major conclusion from our study we think that the drug
5 mixture should no longer be prepared by the anaesthetist but should be provided by the pharmacy
6 or the drug manufacturer so that the risk of under- or overmedication can be avoided.

7

1

2 Acknowledgments

3 None.

4

5 Funding

6 No funding was obtained for this study.

7

8 Conflict of interests

9 All the authors report no conflicts of interest.

10

11 Authors contributions

12 MH: Designed the study, interpreted the data, drafted the manuscript.

13 CS: Performed the chemical and statistical analyses, interpreted the data.

14 PW: Designed the study, interpreted the data, drafted the manuscript.

15 DS: Designed the study, performed the statistical analyses, interpreted the data, drafted the

16 manuscript.

17

1 **REFERENCES**

2

3 1. Rashed AN, Tomlin S, Aguado V, Forbes B, Whittlesea C. Sources and magnitude of error in
4 preparing morphine infusions for nurse-patient controlled analgesia in a UK paediatric hospital. *Int J*
5 *Clin Pharm.* 2016;38:1069-1074.

6 2. Jenkins JG, Khan MM. Anaesthesia for Caesarean section: a survey in a UK
7 region from 1992 to 2002. *Anaesthesia* 2003;58:1114–1118.

8 3. Roofthoof E, Van de Velde M. Low-dose spinal anaesthesia for Caesarean section to prevent
9 spinal-induced hypotension. *Curr Opin Anaesthesiol.* 2008;21:259-262.

10 4. Hunt CO, Naulty JS, Bader AM. Perioperative analgesia with subarachnoid fentanyl-
11 bupivacaine for cesarean delivery. *Anesthesiology.* 1989;71:535-540.

12 5. Aiono-Le Tagaloa L, Butwick AJ, Carvalho B. A survey of peri-operative and postoperative
13 anesthetic practices for cesarean delivery. *Anesthesiol Res Pract.* 2009;2009:510642.

14 6. Parshuram CS, To T, Seto W, Trope A, Koren G, Laupacis A. Systematic evaluation of errors
15 occurring during the preparation of intravenous medication, *CMAJ.* 2008, 178: 42-48.

16 7. Steuer C, Müller U, Haller F, Wiedemeier P. Filling Gaps on Stability Data: Development, Validation
17 and Application of a Multianalyte UHPLC-DAD Method to Determine the Stability of Commonly
18 Administered Drugs in Different Carrier Solutions Used in Palliative Care. *Analytica* 2020; 1: 33-43.

19 8. Schenkel L, Vogel Kahmann I, Steuer C. Opioid-Free Anesthesia: Physico Chemical Stability Studies
20 on Multi-Analyte Mixtures Intended for Use in Clinical Anesthesiology. *Hospital Pharmacy.* May 2021.

21 9. Jasinski T, Migon D, Sporysz K, Kamysz W, Owczuk R. The Density of Different Local Anesthetic
22 Solutions, Opioid Adjuvants and Their Clinically Used Combinations: An Experimental Study.
23 *Pharmaceuticals* 2021,14:801.

24 10. Arzola C, Wieczorek PM. Efficacy of low-dose bupivacaine in spinal anaesthesia for Caesarean
25 delivery: systematic review and meta-analysis. *Br J Anaesth.* 2011;107:308-318.

26

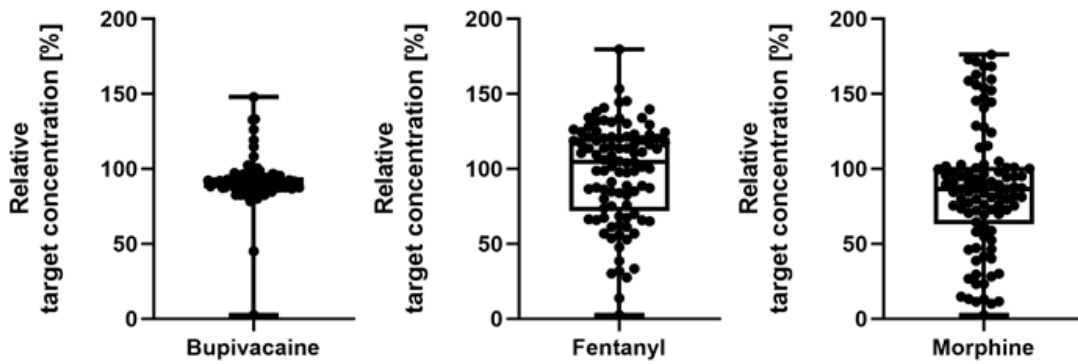
27 11. Bailey PL, Lu JK, Pace NL, Orr JA, White JL, Hamber EA, Slawson MH, Crouch DJ, Rollins DE. Effects
28 of intrathecal morphine on the ventilatory response to hypoxia. *N Engl J Med.* 2000;343:1228-1234.

29 12. Swart M, Sewell J, Thomas D. Intrathecal morphine for caesarean section: an assessment of pain
30 relief, satisfaction and side-effects. *Anaesthesia.* 1997;52:373-377.

31 13. Kato R, Shimamoto H, Terui K, Yokota K, Miyao H. Delayed respiratory depression associated with
32 0.15 mg intrathecal morphine for cesarean section: a review of 1915 cases. *J Anesth.* 2008;22:112-
33 116.

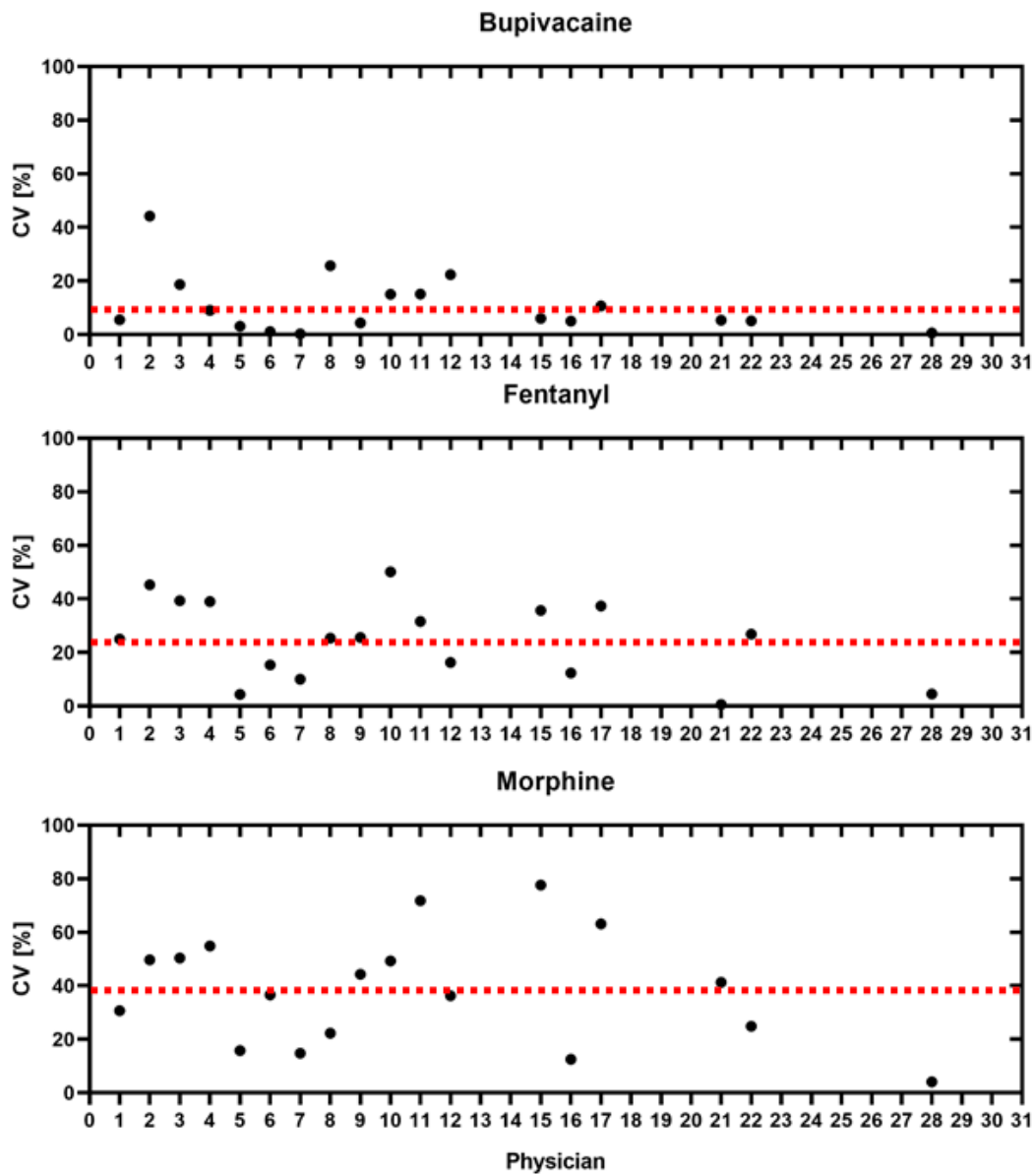
- 1 14.Sharawi N, Carvalho B, Habib AS, Blake L, Mhyre JM, Sultan P. A systematic review evaluating
2 neuraxial morphine and diamorphine-associated respiratory depression after cesarean delivery.
3 *Anesth Analg.* 2018;127:1385–1395.
- 4 15.Bauchat JR, Weiniger CF, Sultan P, Habib AS, Ando K, Kowalczyk JJ, Kato R, George RB, Palmer CM,
5 Carvalho B. Society for Obstetric Anesthesia and Perinatology Consensus Statement: Monitoring
6 Recommendations for Prevention and Detection of Respiratory Depression Associated With
7 Administration of Neuraxial Morphine for Cesarean Delivery Analgesia. *Anesth Analg.* 2019;129:458-
8 474.
- 9 16.Essink-Tjebbes CM, Burger DM, Beelen M, Wuis EW, Hekster YA. Long-term stability of morphine
10 and bupivacaine mixture for spinal use. *Pharm World Sci.* 1999 Jun;21:144-146.
- 11 17.Donnely RF, Wong K, Spencer J. Physical compatibility of high-concentration bupivacaine with
12 hydromorphone, morphine, and fentanyl. *Can J Hosp Pharm.* 2010;63:154-155.

1 figures



2

3 Figure 1: Box-plot representation for individual concentration of selected drugs.



4

1 Figure 2: Intraindividual variations for anesthesiologists preparing more than two mixtures. Dashed
2 line indicates the mean intraindividual variation.

3

4 Table 1. Deviations of observed from intended concentrations of the three drugs.

5

| | Bupivacain [mg/mL] | Fentanyl [µg/mL] | Morphin [µg/mL] |
|---|----------------------------|-----------------------------|-----------------------------------|
| Intended concentration | 4 | 8 | 304 |
| Observed concentration, Mean (SD) [range] | 3.7 (0.49) [1.8-5.9] | 7.9 (2.56) [1.1-14.4] | 265.6 (125.59) [30.7-535.8] |
| % of intended concentration, mean | 92.19% | 98.44% | 87.38% |
| Mean absolute deviation | 0.47 [mg/mL] | 2.09 [µg/mL] | 102.03 [µg/mL] |
| n (%) samples with +/- 0-5% deviation | 20 (20.8%) | 11 (11.5%) | 16 (16.7%) |
| n (%) samples with +/- 5-10% deviation | 29 (30.2%) | 5 (5.2%) | 7 (7.3%) |
| n (%) samples with +/- 10-20% deviation | 41 (42.7%) | 29 (30.2%) | 17 (17.7%) |
| n (%) samples with +/- 20-40% deviation | 4 (4.2%) | 34 (35.4%) | 19 (19.8%) |
| n (%) samples with > +/- 40% deviation | 2 (2.1%) | 17 (17.7%) | 37 (38.5%) |

6

7

8

9