PRACTICE RESEARCH AND INNOVATION

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Development and validation of an HPLC method to determine the stability of fentanyl citrate and bupivacaine hydrochloride mixtures in infusion solutions

Mikołaj Piekarski,[1,2] Anna Jelińska,[1] Kamil Szymczak[1]

[1]Pharmaceutical Chemistry, Karol Marcinkowski University of Medical Sciences, Poznań, Poland
[2]Inhospital pharmacy, Heliodor Święcicki Clinical Hospital of PUMS, Poznań, Poland

Correspondence to
Mikołaj Piekarski, Karol Marcinkowski University of Medical Sciences, Pharmaceutical Chemistry, 6 Grunwaldzka st, Poznań 60-780, Poland; mpiekarski@umed.poznan.pl

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ABSTRACT

Background The use of a combination of different drugs in postoperative analgesia extends the time of analgesia, makes it more efficient and allows the use of lower drug doses, which leads to less risk of side effects and drug dependence. The aim of this study was to develop and validate an HPLC method to determine the stability of fentanyl citrate and bupivacaine hydrochloride mixtures in standard infusion solutions of 0.9% sodium chloride and 5% glucose.

Methods After optimisation, the HPLC method parameters were as follows: LiChrospher 100 CN, 250×4 mm (10 µm) column; mobile phase: mixture of acetonitrile and phosphate buffer at pH 2.8 (3:7, V/V) with addition of 0.08 g/l potassium chloride; flow rate: 1.5 ml/min; column temperature: 30°C; spectrophotometric detection at 210 nm. Development of the method involved checking the impact of acetonitrile and KCl concentrations in the mobile phase and choosing the internal standard. Method validation included determining the specificity of the method, its accuracy, linearity, precision, repeatability, limits of detection and quantification.

Results The retention times of bupivacaine hydrochloride, fentanyl citrate and procaine hydrochloride, used as an internal standard, were approximately 10 min, 15 min and 5 min, respectively. Method validation confirmed its selectivity, accuracy and precision. The average values of the variation and accuracy coefficients were 0.70% and 99.02% for bupivacaine hydrochloride, and 1.76% and 104.53% for fentanyl citrate. The intermediate precision values were 1.25% for bupivacaine hydrochloride and 1.52% for fentanyl citrate.

INTRODUCTION

The management of pain has always been one of the greatest medical challenges. The International Association for the Study of Pain described it as an unpleasant sensory or emotional experience combined with possible or actual tissue damage, or reported while tissues are being damaged.[1] Pain is a physiological symptom which acts as a warning and informs the body about damage or illness, and is therefore essential for life.

Surgical patients are one of the largest group of individuals to experience pain, which is directly related to their treatment. Proper management of postoperative pain allows wounds to heal faster and the patient to recover more quickly. Similarly in child birth, proper administration of analgesia reduces pain and makes delivery less stressful.[2 3]

Continuous analgesia with a mixture of opioids and local anaesthetics is becoming increasingly common for pain management in patients after surgery. It is safe and very effective, and can be successfully used even after severe and extensive abdominal or thoracic interventions.[4 5] Such mixtures are also frequently used in short term analgesia for labour and caesarean section.[6]

Despite great progress in continuous epidural analgesia using mixtures of opioids and local anaesthetics, there are no fast and precise methods to determine the stability and physicochemical safety of such mixtures.

The aim of this study was to develop and validate an HPLC method with UV detection to determine the stability of mixtures of fentanyl citrate and bupivacaine hydrochloride in standard infusion solutions of 0.9% NaCl and 5% glucose.

EXPERIMENTAL

Materials and reagents

Bupivacaine hydrochloride standard was obtained from Sigma-Aldrich (Munich, Germany), while 50 µg/ml fentanyl citrate and 0.5% bupivacaine hydrochloride solutions were obtained from WZF Polfa (Warsaw, Poland) together with fentanyl citrate standard. All others chemicals were obtained from POCh (Gliwice, Poland) and were of HPLC or analytical grade.

Equipment

A Shimadzu chromatographic system (Shimadzu, Kyoto, Japan) consisting of a Shimadzu LC-20AT pump, Shimadzu SPD-20A UV-Vis detector, Shimadzu CTO-10AS UP column thermostat, 20 µl Berkeley 7725i rheodyne and ChroMax 2006 software was used.

Chromatographic conditions

A LiChrospher 100 CN, 250×4 mm, 10 µm particle size (Merck, Darmstadt, Germany) column was used. The final composition of the mobile phase was a mixture of acetonitrile and 0.01 M phosphate buffer at pH 2.81 (3:7, V/V) with addition of 0.08 g/l potassium chloride. The flow rate was 1.5 ml/min, with detection at UV

wavelength 210 nm. The column was kept at 30°C and the injection volume was 20 µl.

Method validation

The method was validated according to the guideline of the International Conference on Harmonization.[7] Microsoft Excel 2007 was used to calculate the method parameters.

Selectivity

The selectivity of the method was examined for samples after preparation and for samples after approximately 72 h storage at room temperature, after the addition of procaine hydrochloride as an internal standard.

Linearity

$P_{fent}/P_{IS}=f(c)$ and P_{bupi}/P_{IS} calibration plots for aqueous solutions of fentanyl citrate and bupivacaine hydrochloride were obtained in the concentration range (2.50–7.52) µg/10 ml for fentanyl citrate and (0.059–0.1770) mg/10 ml for bupivacaine hydrochloride, where P_{fent}/P_{IS} and P_{bupi}/P_{IS} are fentanyl/bupivacaine to procaine hydrochloride (internal standard, IS) peak area ratios. Samples of each solution were injected three times. Calibration curves for an average of each concentration were obtained; each series consisted of five experimental points.

Precision

The precision of the method was determined in relation to repeatability (intra-day). In order to evaluate the repeatability of the method, six samples of bupivacaine hydrochloride solution and fentanyl citrate solution, with the addition of procaine hydrochloride solution as an internal standard, were analysed on the same day. The concentrations of the samples were 111 mg/10 ml for bupivacaine hydrochloride and 0.005 mg/10 ml for fentanyl citrate.

The intermediate precision of the method was also evaluated. In order to evaluate this, samples prepared as before were analysed over the following 2 days.

Accuracy

The accuracy of the method was calculated as a percentage recovery $x_i/\mu \times 100\%$, where x_i is the analysed amount of either fentanyl citrate or bupivacaine hydrochloride in the sample, and µ is the known amount of the substance in the sample. To do so, standard solutions of bupivacaine hydrochloride and fentanyl citrate, and two solutions of drugs containing fentanyl citrate and bupivacaine hydrochloride were prepared, where solutions of the drugs were planned to contain 100% and 80% of the concentration of the standard solutions of each substance, respectively.

Limits of detection (LOD) and quantification (LOQ)

The LOD and LOQ of fentanyl citrate and bupivacaine hydrochloride were calculated from the regression equation as 3.3 S_y/a and 10 S_y/a, respectively, where S_y is the SD and a denotes the slope of the corresponding calibration curve.

Stability studies

Stability studies were performed for mixtures of fentanyl citrate and bupivacaine hydrochloride prepared in solutions of 0.9% NaCl and 5% glucose (B Braun, Melsungen, Germany). From 100 ml bottles of infusion solutions, 30 ml were removed using a Mini Spike system and replace with 20 ml of 0.5% bupivacaine hydrochloride solution and 10 ml of 50 µg/ml fentanyl citrate solution. Bottles with infusion solutions were incubated at 25°C for 15 min prior to addition of the drugs. The prepared mixtures were kept at 25°C for 72 h. At specified intervals, 2 ml samples were taken from the solutions and 1.0 ml from those samples were mixed with 0.2 ml of the internal standard solution. Then, 20 µl of the prepared sample were injected onto the column. In this study solutions of fentanyl citrate and bupivacaine hydrochloride were defined as stable when the substrate loss was not greater than 5% relative to the initial value.

RESULTS AND DISCUSSION

Chromatographic conditions

Selection of the appropriate chromatographic conditions for the determination of the fentanyl citrate and bupivacaine hydrochloride mixtures in standard infusion solutions of 0.9% NaCl and 5% glucose, was based on a literature review of methods for the determination of those substances. The literature recommends using CN-filled columns. Optimal separation of fentanyl citrate and bupivacaine hydrochloride is possible at 15%–40% concentration of the organic component in the mobile phase. It is suggested that the inorganic component should

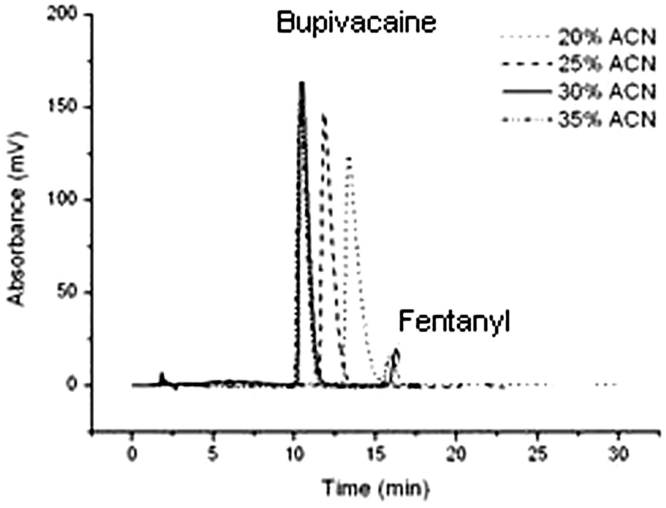

Figure 1 Comparison of mobile phases with different acetonitrile (ACN) concentrations.

Table 1 Comparison of the influence of acetonitrile (ACN) concentration on chromatographic parameters

ACN concentration in the mobile phase	Retention time		Peak symmetry	
	Bupivacaine	Fentanyl	Bupivacaine	Fentanyl
20%	13.32	26.61	4.76	2.17
25%	11.83	20.86	4.21	1.96
30%	10.42	16.26	3.51	1.73
35%	10.36	15.98	3.34	1.66

have pH≤5.[8–13] UV detection at λ=210 nm was selected as it is the absorbance maximum for fentanyl citrate present in the solution at a concentration 200 times lower than that of bupivacaine hydrochloride, which poses a considerable analytical problem.

To optimise the chromatographic conditions, the influence of three parameters was assessed:

- Acetonitrile concentration
- Flow rate
- Concentration of potassium chloride in the mobile phase.

Influence of acetonitrile concentration

Based on the literature, the mobile phase contained acetonitrile and 0.01 M phosphate buffer (pH 2.81). The concentrations of acetonitrile in the mobile phase were 20%, 25%, 30% and 35%. Results obtained using the chromatographic conditions presented in the Chromatographic conditions section are shown in figure 1 and table 1.

Based on those results, the mobile phase with 30% acetonitrile (ACN) was chosen for further method development. The lower ACN concentrations led to lower peak symmetry factors and longer retention times. As the difference between 30% and 35% ACN is not significant, the former was chosen as optimal.

Influence of flow rate

To optimise the flow rate of the mobile phase, four flow rates were analysed: 1.0 ml/min, 1.2 ml/min, 1.5 ml/min and 1.7 ml/min. The results are shown in figure 2 and table 2. The 1.5 ml/min flow rate was chosen for further study. As the difference between the 1.7 and 1.5 ml/min flow rates is not significant, the latter was selected to avoid problems with the internal standard.

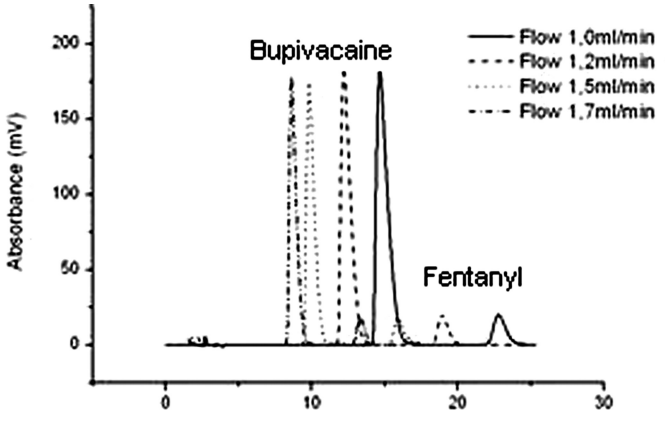

Figure 2 Comparison of different flow rates.

Influence of potassium chloride on chromatographic conditions

To improve the quality of chromatographic separation, the influence of potassium chloride on the mobile phase was investigated. A mobile phase containing 0.08 g/l of potassium chloride was prepared and the results were compared with those obtained without potassium chloride (table 3).

The presence of potassium chloride has a positive effect on the chromatographic separation of bupivacaine and fentanyl.

Method validation

Selectivity and selection of internal standard

In order to avoid injection mistakes, an internal standard – procaine hydrochloride – was used. The retention times for substances were: procaine hydrochloride ~5 min, bupivacaine hydrochloride ~10 min and fentanyl citrate ~15 min. The HPLC method for the determination of bupivacaine and fentanyl was found to be selective in the presence of the internal standard.

Linearity

Linearity was determined separately for bupivacaine hydrochloride and fentanyl citrate. The concentration range was 50%–150% of the concentration of those drugs used in clinical practice: 0.0172–0.0516 mol/l for bupivacaine hydrochloride and 0.473–1.42 mmol/l for fentanyl citrate. Both calibration plots were linear and were described by the equation y=ax; values b calculated from the equation y=ax+b were statistically insignificant, as they were lower than the critical value $t_b=b/S_b$. (table 4).

Precision, accuracy, LOD and LOQ

Precision

To establish precision, the method repeatability of the method was determined. Six samples of the same concentration of both bupivacaine hydrochloride and fentanyl citrate were prepared and injected onto the column. The results are shown in table 5.

Intermediate precision was also established in the same way as repeatability, but samples were injected twice onto the column on 2 different days (table 6).

Results show that the method has good precision.

Table 2 Comparison of the influence of flow rate on chromatographic parameters

	Retention time		Peak symmetry	
Flow rate	Bupivacaine	Fentanyl	Bupivacaine	Fentanyl
1.0 ml/min	14.66	22.78	2.96	1.65
1.2 ml/min	12.19	18.92	2.93	1.64
1.5 ml/min	9.81	15.46	2.72	1.73
1.7 ml/min	8.63	13.33	2.67	1.57

Table 3 Influence of potassium chloride on chromatographic separation

	Retention time		Peak symmetry	
Mobile phase	Bupivacaine	Fentanyl	Bupivacaine	Fentanyl
30% ACN	10.15	15.46	3.51	1.73
30% ACN with 0.08 g/l KCl	9.77	15.10	3.38	1.70

ACN, acetonitrile.

Table 4 Statistical parameters for linearity of bupivacaine hydrochloride and fentanyl citrate

Bupivacaine hydrochloride	Fentanyl citrate
y=ax±b	y=ax±b
a±Δa=53134±0.521	a±Δa=0.031±0.002
b±Δb=0.015±0.065	b±Δb=0.006±0.008
Sa=0.246	Sa=0.001
Sb=0.030	Sb=0.004
Sy=0.040	Sy=0.005
Ta=216184	Ta=43 313
Tb=0.476	Tb=1542
R=0.9998	R=0.9958
y=ax	y=ax
a±Δa=53245±0.505	a±Δa=0.033±0.001
Sa=0.238	Sa=0.001
Sy=0.039	Sy=0.005
r=0.9998	r=0.9958

S is SD and T is variance.

Table 5 Method precision for bupivacaine hydrochloride and fentanyl citrate

Bupivacaine hydrochloride	
Average	6.2635
Variance: s^2	1.89×10^{-3}
SD: s	0.04354
Relative SD: S_y	0.00695
Coefficient of variation: W_z	0.70%
Fentanyl citrate	
Average	0.1666
Variance: s^2	8.63×10^{-6}
SD: s	0.00294
Relative SD: S_y	0.01763
Coefficient of variation: W_z	1.76%

Table 6 Intermediate method precision for bupivacaine hydrochloride and fentanyl citrate

Bupivacaine hydrochloride	
Average	6.3284
Variance: s^2	6.27×10^{-3}
SD: s	0.0792
Relative SD: S_y	0.0125
Coefficient of variation: W_z	1.25%
Fentanyl citrate	
Average	0.1665
Variance: s^2	6.38×10^{-6}
SD: s	0.0025
Relative SD: S_y	0.0152
Coefficient of variation: W_z	1.52%

Table 7 Validation parameters of the method for bupivacaine hydrochloride and fentanyl citrate

Parameter	Bupivacaine	Fentanyl
Linearity range	0.059–0.177 mg/ml	2.5–7.25 µg/ml
LOD	2.47×10^{-3} mg/ml	0.5 µg/ml
LOQ	7.5×10^{-3} mg/ml	1.5 µg/ml
Repeatability	0.70%	1.76%
Intermediate precision	1.25%	1.52%
Percentage recovery	99.02%	104.53%

LOD, limit of detection; LOQ, limit of quantification.

and fentanyl citrate were prepared and two solutions of each substance at drug concentrations 100% and 80% of the standard solution.

Limits of detection and quantification

The LOD and LOQ for fentanyl citrate and bupivacaine hydrochloride were calculated from the regression equations 3,3 S_y/a and 10 S_y/a, respectively, where S_y is the SD and a denotes the slope of the corresponding calibration curve (table 7).

Table 8 Stability of bupivacaine hydrochloride and fentanyl citrate in 0.9% NaCl, 25°C

Sample	t (h)	PIS	PBup	PFent	PBup/PIS	%B	PFent/PIS	%F
1	0	888999	30473943	171402	342789	100.00%	0.1928	100.00%
2	1	870079	29416332	164967	338088	98.63%	0.1896	98.34%
3	6.5	785461	27937316	154065	355680	103.76%	0.1961	101.73%
4	23	862758	29865464	167883	346163	100.98%	0.1946	100.93%
5	32.5	544133	19132839	106660	351621	102.58%	0.1960	101.67%
6	47	889441	30055613	166951	337916	98.58%	0.1877	97.35%
7	51.5	605950	21098765	118155	348193	101.58%	0.1950	101.13%
8	72	881896	29663404	166695	336359	98.12%	0.1890	98.04%
				Bupivacaine hydrochloride		Fentanyl citrate		
Average amount				100.53%		99.89%		
SD				2.05		1.75		

Accuracy

Method accuracy is defined as recovery percentage ($x_i/\mu\times100\%$, where x_i is the analysed amount of either fentanyl citrate or bupivacaine hydrochloride in the sample, and µ is the known amount of the substance in the sample). Standard solutions of bupivacaine hydrochloride

Stability studies

The results of stability studies are presented in tables 8 and 9. As the results show that neither of the solution concentrations falls below the 95% starting concentration limit, we considered both solutions stable. This corresponds with previous studies.[10 12 14]

Table 9 Stability of bupivacaine hydrochloride and fentanyl citrate in 5% glucose, 25°C

Sample	t (h)	PIS	PBup	PFent	PBup/PIS	%B	PFent/PIS	%F
1	0	927521	28908568	155979	3116756	100.00%	0.1681	100.00%
2	1	890054	28720334	150732	3226808	103.53%	0.1693	100.70%
3	6.5	808511	25436765	137987	3146125	100.94%	0.1706	101.49%
4	23	904547	28270820	156270	3125412	100.28%	0.1727	102.73%
5	24	909636	28961765	160160	3183885	102.15%	0.1760	104.70%
6	47	999233	30018765	164415	3004181	96.39%	0.1645	97.84%
7	48	898613	28241824	155028	3142824	100.84%	0.1725	102.59%
8	72	921833	28846309	155120	3129234	100.40%	0.1682	100.06%
				Bupivacaine hydrochloride		Fentanyl citrate		
Average amount				100.57%		101.26%		
SD				2.05		2.10		

CONCLUSION

The proposed method was proved to be suitable for determining the stability of fentanyl citrate and bupivacaine hydrochloride mixtures in standard intravenous fluids. It may be applied to establish the stability of drug mixtures prepared in the hospital pharmacy using an automatic BAXA compounder. Further studies on fentanyl and bupivacaine regarding the microbiological purity of their mixtures are necessary.

Competing interests None.

Provenance and peer review Not commissioned; externally peer reviewed.

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