

Validation Study of UPLC Method for Determination of Morphine, Ropivacaine and Ziconotide in Combination for Intrathecal Analgesia

Rossignol E^{1,2}, Sorrieu J^{1*}, Beaussart H¹, Kieffer H¹, Folliard C¹, Dupouiron D³ and Devys C¹

¹Institut de Cancérologie de l'Ouest Paul Papin-Pharmacy, Angers, France

²Institut de Cancérologie de l'Ouest René Gauducheau-Biology, Saint Herblain, France

³Institut de Cancérologie de l'Ouest Paul Papin-Anesthesia and Pain department, Angers, France

Abstract

Pain is often considered as the most feared symptom amongst individuals living with cancer. It can arrive at any stage during the course of the illness. In 15% to 20% of patients, conventional analgesic therapy either fails to relieve pain or induces adverse effects. Use of analgesic admixture has been recommended by the most recent consensus conferences. Several studies found evidence of synergistic effects of intrathecal analgesic admixtures, most notably these containing ziconotide, morphine and ropivacaine, administering by a fully implantable pumps. The refills were prepared under a laminar airflow hood under strictly aseptic conditions, by the hospital pharmacist. This group of drugs that commonly used can be at the origin of errors inducing adverse effects in patients. In order to evaluate the accuracy of compounding of intrathecal admixtures, a new analytical method by simple liquid chromatography-ultraviolet spectrometry method was developed and validated for the simultaneous quantification of three analgesic drugs (morphine, ropivacaine and ziconotide). The method was validated according to the recommendation of the US Food and Drug Administration (FDA). The method was linear, between 0.1 to 4 µg/ml for ziconotide, 0.1 to 10 mg/ml for ropivacaine and 0.1 to 32 mg/ml for morphine. Forced degradation of ziconotide by acidic conditions allowed formation and detection of degradation products by the analytical method. This method can be considered as a stability indicating method. It is also part of a continuing quality process designed to improve accuracy of preparation.

Keywords: Intrathecal analgesia; Morphine; Ropivacaine; Ziconotide; UPLC-UV

Introduction

The management of cancer is not limited to the treatment of the disease but must also consider patient pain. In 15% to 20% of cancer patients, conventional analgesic therapy recommended by WHO fails to relieve pain [1]. In France, Intrathecal Analgesia (IA) is recommended by French Society of Anesthesia and Intensive Care Medicine (SFAR) in case of refractory chronic cancer pain, as well as in patients with treatment-related adverse effects impairing patient's quality of life [2]. The administration of anesthetic drugs in the central nervous system can achieve similar or better analgesic results and can be easier to use for clinicians [3-5].

The administration of these analgesic drugs by intrathecal route is done by using a pump (an internal implantable or external intrathecal pump), connected with a tunneled catheter implanted in the intrathecal area [6,7]. The implantable pump has a reservoir which has to be refilled. External pumps have a bag containing the anesthetic substances that needs to be regularly replaced. This refill is prepared in a controlled atmosphere area under strict aseptic conditions in a vertical laminar flow cabinet by manipulators trained on these preparations to be used internally. Commercial pharmaceutical products are used for preparation.

Over the years, various molecules have been used in combination for IA. Morphine remains the reference in case of strong to intense pain. Ropivacaine or bupivacaine are used to strengthen morphine effects by blocking nerve conduction in the sodium channels. Ziconotide is a small ω -conotoxine peptide isolated from a marine cone snail, *Conus magus* and acts as a selective blocker of voltage-sensitive calcium channels of N-type [8-11]. Due to its narrow therapeutic range and its potentially serious adverse effects (psychiatric and nervous system disorders), this drug is only indicated for the treatment of severe, chronic pain in adults who require intrathecal (IT) analgesia [12,13].

Finally, clonidine, sufentanil, ketamine can also be used, although these molecules are not present in the last International recommendations.

The Cancerology Center Paul Papin protocol includes an association of morphine, ropivacaine and ziconotide in Intrathecal preparations (PIT). Concentrations of each analgesic agent vary from one preparation to another because they are adapted for each patient's pain evaluated by the physician before the prescription [14]. Concentrations mean and range are listed in the Table 1.

Dosage errors most likely explain a significant percentage of the side effects [15,16] caused by ziconotide. Ziconotide is a particularly powerful drug. Its therapeutic index is low and adverse effects may occur at low doses (1 µg/day) [8,14,17]. The accuracy of the injected dose is therefore vital and no other clinical study has checked concentrations of ziconotide really infused.

To secure the preparations, a systematic analytical control must be performed before release. Over time, several authors have studied the ziconotide dosage in combination with morphine and ropivacaine by using methods of liquid chromatography combined with ultra-violet detection, although ziconotide concentrations were higher than those that we are actually using (25 µg/mL) [18-20].

***Corresponding author:** Sorrieu J, Institut de Cancérologie de l'Ouest Paul Papin-Pharmacy, Angers, France, Tel: 0241352721; Fax: 0241352866; E-mail: jeremy.sorrieul@ico.unicancer.fr

Received March 10, 2016; **Accepted** March 21, 2016; **Published** March 28, 2016

Citation: Rossignol E, Sorrieu J, Beaussart H, Kieffer H, Folliard C, et al. (2016) Validation Study of UPLC Method for Determination of Morphine, Ropivacaine and Ziconotide in Combination for Intrathecal Analgesia. J Anal Bioanal Tech 7: 310. doi:10.4172/2155-9872.1000310

Copyright: © 2016 Rossignol E, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Mean concentrations [Min-Max]	
Morphine	3.34 [0.05-23.9] mg/mL
Ropivacaine	7.54 [0.4-9.8] mg/mL
Ziconotide	0.98 [0.04-5.8] µg/mL

Table 1: Mean concentrations of morphine, ropivacaine and ziconotide in the PIT.

The aim of this study was to develop and validate a new method of determination of ziconotide, morphine and ropivacaine using ultra-performance liquid chromatography (UPLC) combined with an ultraviolet detector in order to control our preparation according to national and international recommendations [21-23].

Materials and Methods

Analytes and reagents

For development and validation of the method the commercial pharmaceutical products were used. Morphine (CDM Lavoisier, La Chaussée-Saint Victor, France) is available under a ready-to-use intravenous and intrathecal formulation of morphine sulfate 500 mg/10 mL including the excipients sodium chloride, hydrochloric acid and water for injection.

Ropivacaine (Fresenius Kabi, Sèvres, France) is supplied in 20 mL bottles containing 200 mg and the excipients sodium chloride, hydrochloric acid, sodium hydroxide and water for injection. Ziconotide (Prialt®, Eisai SAS, Paris La Défense, France) is provided in 100 µg/1 mL bottles, including the excipients methionine, sodium chloride, hydrochloric acid, sodium hydroxide and water for injection. The three drugs are mixed together, alone or with a saline solution of 0.9% as excipient (Fresenius Kabi, Sèvres, France), before refilling the intrathecal pump of 40 mL.

Acetonitrile and trifluoroacetic acid were supplied by VWR (Fontenay aux Roses, France) and ultrapure water was obtained from Elga Purelab DV25 system. Solvents are HPLC-MS/MS grade to avoid impurities like metal ions in the mobile phase.

Chromatographic and UV detection conditions

Analyses were conducted using an UPLC Acquity® H-Class® interfaced to a photodiode array detector (PDA) (Waters, Guyancourt, France). The column used was an Acquity UPLC® BEH C18 1.7 µm 2.1 × 50 mm heated at 50°C and the mobile phase was a gradient of ultrapure water with 0.1% trifluoroacetic acid and acetonitrile with 0.1% trifluoroacetic acid. Two liquid chromatography (LC) methods were developed due to concentration range difference between analytes. Respective gradients are presented in Tables 2 and 3. For morphine and ropivacaine analysis, 0.3 µL were injected into the column whereas 10 µL were injected for ziconotide. Detection was monitored at 285 nm for morphine, at 230 nm for ropivacaine and at 200 nm for ziconotide. In these conditions, the retention times were of 0.62 minutes, 1.99 minutes and 1.62 minutes, respectively.

Standards and quality controls (QC) preparations

Standards of morphine (0.1 to 32 mg/mL) and ropivacaine (0.312 to 10 mg/mL) were prepared by serial dilution of the pharmaceutical product with NaCl 0.9% in glass vials. Ziconotide standards (0.1 to 4 µg/mL) were individually prepared by diluting the commercial solution in polypropylene vials to avoid wrong results when using serial dilution.

Quality controls (QC) containing a mean concentration of the three active substances (Morphine 4 mg/mL, Ropivacaine 8 mg/mL and ziconotide 1 µg/mL) in NaCl 0.9% were prepared and analyzed during each validation run.

Validation procedure

The validation procedure was performed according to the US Food and Drug Administration (FDA) and Groupe d'Evaluation et de Recherche sur la Protection en Atmosphère Contrôlée (GERPAC) recommendations [22,23].

Selectivity: Selectivity with regard to excipients or other constituents of the mixture has been checked by analyzing individually three samples containing only the excipient (NaCl 0.9%) and the two other active substances in a mean concentration.

Calibration curve: The response of the instrument with regard to the concentration was studied. Concentrations were chosen on the basis of the expected range. For each analyte, six calibration standards and one zero standard have been analyzed during 3 runs (N=3), performed on three days by different manipulators.

Calibration curves were using a linear regression of the calibration points excluding the zero standards. Weighting and adequacy of the regression model was determined by statistical analysis (Levene's test and lack-of fit test) according to the FDA recommendations [22]. The simplest model was used and between-run accuracy and precision of the standards were calculated.

QC within-run and between-run accuracy and precision: The accuracy and precision of the methods were evaluated by analyzing six individually prepared QC during each run. Within-run accuracy and precision were determined without reagent or manipulator variability in a single run (n=6) whereas between-run precision and accuracy were determined on three runs (total: n=18) performed in different days and involving several manipulators.

Accuracy was expressed as relative error (RE %) compared with nominal values and precision (repeatability) was determined by calculating the relative standard deviation (RSD %) of the measured QC concentration. Limits of acceptable were fixed at ±15% deviation from the expected for RSD and RE.

Carryover: Blanks containing 0.9% sodium chloride without analyte were injected after three high concentration samples (QC

Time (min)	Flow Rate (mL/min)	% Solvent A	% Solvent B
0	0.5	95	5
0.5	0.5	90	10
2.5	0.5	25	75
2.8	0.5	0	100
3.8	0.5	0	100
4.5	0.5	95	5
5.5	0.5	95	5

Table 2: Gradient used for morphine and ropivacaine LC analysis.

Time (min)	Flow Rate (mL/min)	% Solvent A	% Solvent B
0	0.5	95	5
0.5	0.5	90	10
0.9	0.5	75	25
2.0	0.5	75	25
2.5	0.5	0	100
3.5	0.5	0	100
3.8	0.5	95	5
5.5	0.5	95	5

Table 3: Gradient used for ziconotide LC analysis.

samples) in order to evaluate carry over for each analyte. This injection sequence was repeated 3 times.

Forced degradation study: As far as it was possible, a degradation of around 20% of the active substance was carried out by treating the analyte diluted in NaCl 0.9% with 0.5% hydrochloric acid followed by neutralization with 0.5% of sodium hydroxide. Each degradation product was identified by its retention time relative to the main analyte and mass balance was determined.

Results and Discussion

Selectivity

No interfering peaks were detected at the specific retention times of the three analytes of interest in the tested samples. The absence of interference by 0.9% NaCl and the two other active substances of the mixture have been validated for the three analytes. Thus, the matrix effect was not studied.

Response function analysis

Morphine and ziconotide, equality of variances was rejected and calibration curves were linear over the studied concentration ranges with $1/x$ regression. For ropivacaine, homogeneity of variances was accepted according to the Levene's Test (calculated $F=2.932 < \text{theoretical } F=3.106$, IC 95%), so the linear regression was carried out without weighting. Calibrators calculated concentrations and between-run accuracy and precision are presented in Table 4. Acceptance criteria were met with absolute RE and RSD $< 15\%$ for the three analytes. The regression coefficient (R^2) for each calibration curve was > 0.996 .

QC within-run and between run accuracy and precision

Within-run and between-run accuracy and precision were determined with QC samples as described in the validation procedure section. Within-run precision ranged between -7.0% and 2.4% for ziconotide, -0.7% and -1.6% for ropivacaine, -0.2% and -0.1% for morphine. Within-run and between accuracy and precision met the acceptance criteria with RSD $< 15\%$ and inaccuracy ranged from -15% to $+15\%$ for the three studied molecules (Table 5).

Carryover

No peak was detected in the blank series, meaning that no intersample contamination occurred for ziconotide, morphine and ropivacaine.

Degradation study

Ziconotide ($1.3 \mu\text{g/mL}$) was degraded by adding 0.5% HCl to the final concentration. The vial was opened during 3 hours in order to mimic a prolonged exposition to oxygen and allow oxidation reactions representing 20.54% of the initial compound was observed at a relative retention time of 0.95 minutes (Figure 1). The mass balance obtained was 100.1%, which proves that no other degradation product was found (Table 6). This degradation product has a specific retention time of 1.555 minutes and will be therefore visible in samples containing morphine and ropivacaine.

No degradation product was observed for morphine or ropivacaine despite treatments with hydrochloric acid, hydrogen peroxide, sodium hydroxide or UV.

Application

After validation, the method has been routinely used for PIT preparation analysis. During the first month 248 PIT mixtures

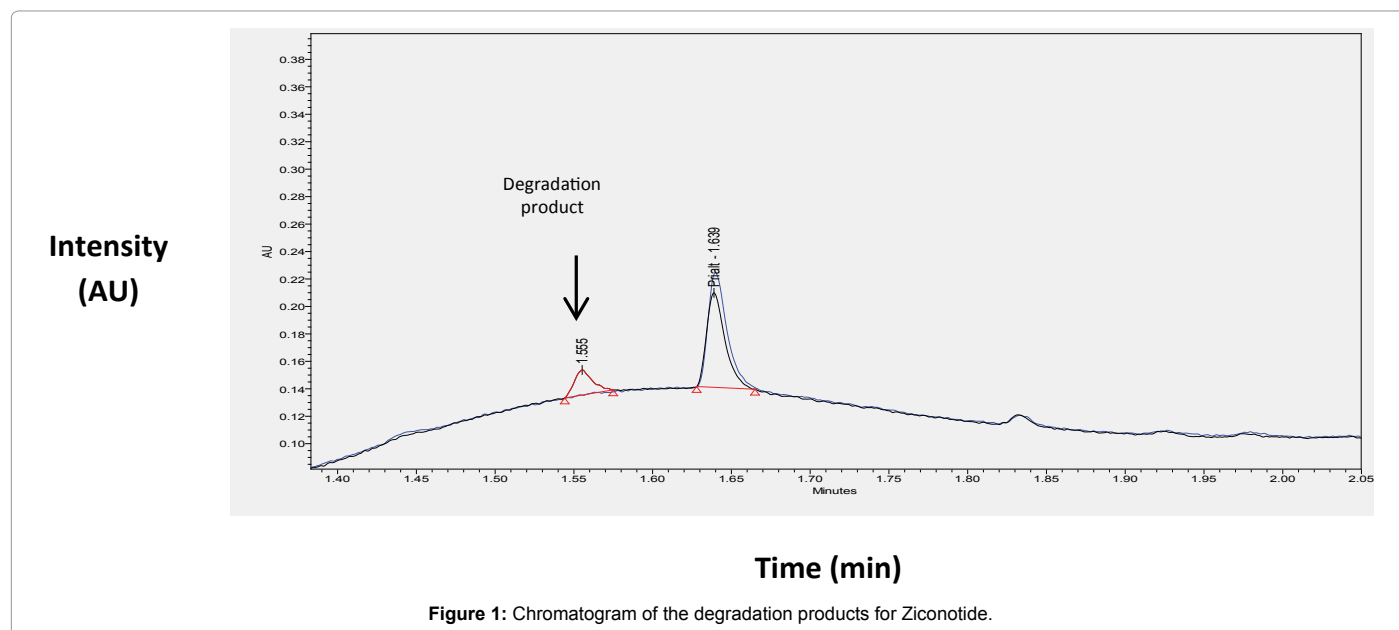
(including 224 with ziconotide) have been prepared and analyzed before release. Samples with concentrations out of range were diluted. The acceptance criteria were $\pm 10\%$ for morphine and ropivacaine and $\pm 15\%$ for ziconotide. The absolute RE means were between 2.1% and 3.2% for morphine, between 0.9% and 3.1% for ropivacaine and between 5.6% and 9.3% for ziconotide (Table 7). One preparation has been rejected (absolute RE -22.80%).

Conclusion

Intrathecal Analgesia is in progression and our activity is increasing with a progression of 54% between 2012 and 2013. A method based on UPLC-UV to quantify admixture with morphine, ropivacaine and ziconotide was developed and fully validated according to FDA recommendations. The calibration range was from 0.1 to $4 \mu\text{g/mL}$ for ziconotide, 0.316 to 10 mg/mL for ropivacaine and 0.1 to 32 mg/mL for morphine. This method offers an interesting compromise in terms of analysis time and detection level compared with previously published methods [18,20]. This quantitative analysis limits dosage errors and overdosing, particularly for ziconotide. The method for ziconotide, morphine and ropivacaine determination that we developed was

Nominal concentrations	Calculated concentrations			Between-run accuracy and precision		
	Run 1	Run 2	Run 3	mean	RE%	RSD%
Morphine calibrators (mg/mL)						
0.10	0.090	0.101	0.099	0.097	-3.2%	6.0%
0.50	0.519	0.502	0.499	0.507	1.3%	2.1%
1.00	1.041	1.001	1.000	1.014	1.4%	2.3%
4.00	4.091	3.924	4.016	4.010	0.3%	2.1%
16.00	16.051	16.073	16.174	16.100	0.6%	0.4%
32.00	31.808	31.998	31.811	31.872	-0.4%	0.3%
R^2	0.9999	0.9999	0.9999	0.9999		0.001%
Slope	0.9939	1.0010	0.9961	0.9970		0.361%
Ropivacaine calibrators (mg/mL)						
0.312	0.285	0.302	0.306	0.298	-4.6%	3.8%
0.625	0.606	0.619	0.619	0.615	-1.6%	1.2%
1.25	1.245	1.245	1.244	1.244	-0.4%	0.1%
2.50	2.529	2.510	2.508	2.516	0.6%	0.5%
5.00	5.054	5.023	5.023	5.033	0.7%	0.4%
10.00	9.969	9.987	9.988	9.981	-0.2%	0.1%
R^2	0.9999	0.9999	0.9999	0.9999		0.002%
Slope	1.0000	1.0000	1.0000	1.0000		0.0%
Ziconotide calibrators ($\mu\text{g/mL}$)						
0.10	0.108	0.110	0.102	0.107	6.8%	3.7%
0.20	0.206	0.217	0.211	0.211	5.6%	2.6%
0.40	0.375	0.362	0.364	0.367	-8.3%	2.0%
1.00	0.919	0.943	0.981	0.948	-5.2%	3.3%
2.00	2.027	1.810	2.087	1.975	-1.3%	7.4%
4.00	4.064	4.258	3.955	4.092	2.3%	3.8%
R^2	0.9997	0.9969	0.9995	0.9987		0.2%
Slope	1.0195	1.0517	0.9968	1.0227		2.7%

Table 4: Calibrators between-run accuracy and precision.



	Nominal Concentration	Calculated Concentrations			Between-run accuracy and Precision		
		Run 1	Run 2	Run 3	mean	RE%	RSD%
Morphine					3.994	-0.1%	0.7%
QC1	4.0 mg/mL	3.966	3.997	3.999	3.994	-0.1%	0.7%
QC2		4.012	3.976	4.015			
QC3		4.026	3.973	3.973			
QC4		3.954	4.002	3.940			
QC5		3.993	4.033	4.028			
QC6		4.035	3.985	3.986			
Within-run mean		3.998	3.994	3.990			
Within-run RE% mean	-0.1%	-0.1%	-0.2%				
Within-run CV%	0.8%	0.6%	0.8%				
Ropivacaïne							
QC1	8.0 mg/mL	7.905	7.844	7.935	7.937	-0.8%	0.9%
QC2		7.971	7.859	8.037			
QC3		7.965	7.858	7.978			
QC4		7.859	7.834	7.994			
QC5		7.994	7.946	7.962			
QC6		7.988	7.874	8.063			
Within-run mean		7.947	7.869	7.995			
Within-run RE% mean	-0.7%	-1.6%	-0.1%				
Within-run RSD%	0.7%	0.5%	0.6%				
Ziconotide							
QC1	1.0 µg/mL	1.021	0.987	0.879	0.985	-1.5%	5.0%
QC2		1.065	1.003	0.921			
QC3		1.073	0.993	0.941			
QC4		1.026	1.012	0.948			
QC5		0.981	0.999	0.949			
QC6		0.978	1.010	0.943			
Within-run mean		1.024	1.001	0.930			
Within-run RE% mean	2.4%	0.1%	-7.0%				
Within-run RSD%	3.9%	1.0%	2.9%				

Table 5: Within-run and between-run accuracy and precision obtained for QC samples.

	Response (Peak area)	Retention time (RT in min)	Relative retention time (RRT in min)	% Area	Mass balance (%)
Peak area analyte non degraded	71436	1.640	1.00	100.00	-
Peak area analyte - degraded	56834	1.639	1.00	79.559	100.1%
Peak area of product of degradation 1	14692	1.555	0.95	20.567	

Table 6: Degradation products of ziconotide.

Concentration range	RE % Min / Max	Absolute RE mean (%)	No of preparations
Morphine (mg/mL)			
0.1-2.5	-4.60% / 9.95%	2.0%	122
2.5-5.0	-5.55% / 8.93%	2.1%	50
5.0-10.0	-6.03% / 3.77%	2.4%	43
>10.0	-7.09% / 0.43%	3.2%	31
Total Range	-7.09% / 9.95%	2.3%	246
Ropivacaine (mg/mL)			
0.316-2.5	-7.00% / 2.25%	3.1%	14
2.5-5.0	-2.40% / 3.07%	1.3%	13
5.0-7.5	-3.56% / 4.28%	1.4%	35
>7.5	-7.78% / 2.95%	0.9%	181
Total Range	-7.78% / 4.28%	1.1%	243
Ziconotide (µg/mL)			
0.1-0.25	-14.00% / 14.00%	8.7%	23
0.25-0.5	-10.59% / 13.33%	6.1%	40
0.5-0.75	-22.80% / 13.15%	6.9%	73
>0.75	-14.75% / 11.13%	5.6%	86
Total Range	-22.80% / 14.00%	6.4%	222

Table 7: Absolute RE mean obtained for routinely analyzed PIT preparations.

validated, used in routine and can be consider as a stability indicating method. In order to determine the expiration date, a stability study will be carried out. Results may allow us to develop subcontracting activity which would consist in preparations shipment in other centers.

References

- Meuser T, Pietruck C, Radbruch L, Stute P, Lehmann KA, et al. (2001) Symptoms during cancer pain treatment following WHO-guidelines: a longitudinal follow-up study of symptom prevalence, severity and etiology. *Pain* 93: 247-257.
- AFSOS (2014) Référentiels en soins oncologiques de support - Prise en charge de la douleur? antalgie intrathécale.
- Deer TR, Smith HS, Burton AW, Pope JE, Doleys DM, et al. (2011) Comprehensive consensus based guidelines on intrathecal drug delivery systems in the treatment of pain caused by cancer pain. *Pain Physician* 14: E283-312.
- Deer TR, Prager J, Levy R, Rathmell J, Buchser E, et al. (2012) Polyanalgesic Consensus Conference 2012: recommendations for the management of pain by intrathecal (intraspinal) drug delivery: report of an interdisciplinary expert panel. *Neuromodulation* 15: 436-464.
- Deer TR (2012) Polyanalgesic Consensus Conference 2012. *Neuromodulation* 15: 418-419.
- Bhatia G, Lau ME, Koury KM, Guler P (1000) Intrathecal Drug Delivery (ITDD) systems for cancer pain. Version 4.
- Bottros MM, Christo PJ (2014) Current perspectives on intrathecal drug delivery. *J Pain Res* 7: 615-626.
- Dupoiron D, Bore F, Lefebvre-Kuntz D, Brenet O, Debourmont S, et al. (2012) Ziconotide adverse events in patients with cancer pain: a multicenter observational study of a slow titration, multidrug protocol. *Pain Physician* 15: 395-403.

- Schmidtko A, Lötsch J, Freynhagen R, Geisslinger G (2010) Ziconotide for treatment of severe chronic pain. *Lancet* 375: 1569-1577.
- Mathur VS (2000) Ziconotide: A new pharmacological class of drug for the management of pain. *Journal of Critical Care* 19: 67-75.
- Vink S, Alewood PF (2012) Targeting voltage-gated calcium channels: developments in peptide and small-molecule inhibitors for the treatment of neuropathic pain: VGCC ligands and pain. *Br J Pharmacol* 167: 970-989.
- European Medicine Agency (EMA) Ziconotide approval.
- Alicino I, Giglio M, Manca F, Bruno F, Puntillo F (2012) Intrathecal combination of ziconotide and morphine for refractory cancer pain: A rapidly acting and effective choice. *Pain* 153: 245-249.
- Dupoiron D, Devys C, Bazin C, Folliard C, Lebrec N, et al. (2015) Rationale for Prospective Assays of Intrathecal Mixtures Including Morphine, Ropivacaine and Ziconotide: Prevention of Adverse Events and Feasibility in Clinical Practice. *Pain Physician* 18: 349-357.
- Rauck RL, Wallace MS, Leong MS, Minehart M, Webster LR, et al. (2006) A Randomized, Double-Blind, Placebo-Controlled Study of Intrathecal Ziconotide in Adults with Severe Chronic Pain. *J Pain Symptom Manage* 31: 393-406.
- Staats PS, Yearwood T, Charapata SG, Presley RW, Wallace MS, et al. (2004) Intrathecal Ziconotide in the Treatment of Refractory Pain in Patients with Cancer or AIDS: A Randomized Controlled Trial. *JAMA* 291: 63-70.
- Maier C, Gockel HH, Gruhn K, Krumova EK, Edel MA (2011) Increased risk of suicide under intrathecal ziconotide treatment? - a warning. *Pain* 152: 235-237.
- Shields D, Montenegro R (2007) Chemical stability of ziconotide-clonidine hydrochloride admixtures with and without morphine sulfate during simulated intrathecal administration. *Neuromodulation* 10 Suppl 1: 6-11.
- Shields DE, Liu W, Gunning K, Montenegro R (2008) Statistical evaluation of the chemical stability of ziconotide solutions during simulated intrathecal administration. *J Pain Symptom Manage* 36: e4-6.
- Shields D, Montenegro R, Ragusa M (2005) Chemical stability of admixtures combining ziconotide with morphine or hydromorphone during simulated intrathecal administration. *Neuromodulation* 8: 257-263.
- EMA (2011) Guideline on bioanalytical method validation.
- FDA (2013) Bioanalytical Method Validation-Guidance for industry.
- Sautou V, Brossard D, Chedru-Legros V, Crauste-Manciet S, Fleury-Souverain S, et al. (2013) Methodological guidelines for stability studies of hospital pharmaceutical preparations. *GERPAC*.