Development and validation of an assay method for Tramadol and Nefopam in solution to study the stability of experimental drugs

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INTRODUCTION

The Drug Preparation Unit was asked to manufacture treatment units (TU) for an institutional clinical study evaluating multimodal analgesic management with **Tramadol, Nefopam, Ketamine and Remifentanil** in mechanically ventilated intensive care patients. Inclusion must be carried out within 24 hours of intubation, and the **treatment duration was 72 hours**, either a **production of 43 TU per patient**. In view of the time required for inclusion and the production volume, the **manufacture of kits for 72h of treatment** was envisaged. Stability data in the literature at the desired concentrations for Ketamine and Remifentanil were sufficient (over 14 days), unlike those for **Nefopam (48h[1])** and **Tramadol (14 days[2])**.

AIM

Develop and validate a **common stability-indicating assay method** for solutions of :

- **Tramadol** *(in polypropylene syringe)* : 10.3 mg.ml-1
- **Nefopam** *(in polypropylene syringe)* : 2.5 mg.ml-1
- **Mixture of Tramadol** (0.943 mg.ml^{-1}) **+ Néfopam** (0.377 mg.ml^{-1}) in 0.9% sodium chloride solution *(polyolefin bag)*.

MATERIALS & METHODS

The analytical method developed was performed by HPLC :

HPLC characteristics

Stationary phase	*Mobile phase*	*Pump flow rate*	*Injection volume*
Column Gravity C18 152x4,6 mm, 5µm	• 80% phosphate buffer (pH 3) • 20% acetonitrile	2 ml.min^{-1}	10 µl

Analytical validation parameters

Calibration range	*Tramadol + Nefopam in mixture*	***Tramadol*** : 0.6 to 1.35 mg.ml^{-1} ***Nefopam*** : 0.125 to 0.5625 mg.ml^{-1}
Repeatability	**6 replicates of the assay** for each product *Tramadol / Nefopam / Tramadol + Nefopam mixture*	
Intermediate fidelity	**6 runs** (alternating manipulators between each run) : ***1 run/day - 3 separate manipulators***	
Forced degradation	*Acid hydrolysis*	**1N HCl, 60°C, 4h**
	Basic hydrolysis	**1N NaOH, 60°C, 4h**
	Oxidation	**H2O2 à 3%, 60°C, 4h**
	UV light	**365 nm, 4h**

RESULTS

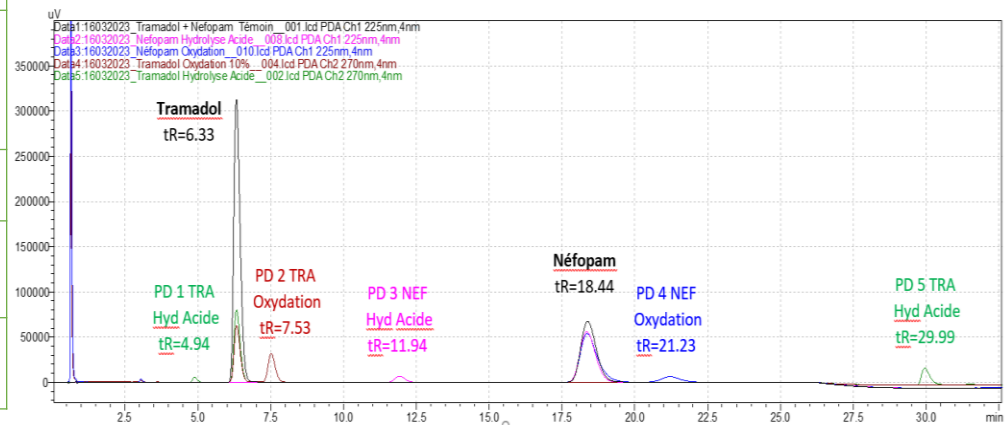
Analytical validation

	TRAMADOL		NEFOPAM	
Retention time	**6,33 min**		**18,44 min**	
Correlation coefficient	R > 0,999		R > 0,999	
	Only 10,3 mg.ml^{-1}	*In mixture* 0.943 mg.ml^{-1}	*Only* 2.5 mg.ml^{-1}	*In mixture* 0.377 mg.ml^{-1}
Mean concentration *(mg/ml ± standard deviation)*	10,374 ± 0,110	0,950 ± 0,005	2,491 ± 0,014	0,376 ± 0,001
Repeatability *(CVr)*	**0,45 %**	**0,04 %**	**0,32 %**	**0,07 %**
Intermediate fidelity *(CVfi)*	**1,14 %**	**0,60 %**	**0,58 %**	**0,37 %**
Accuracy % *(± standard deviation)*	100,72 ± 1,07	100,70 ± 0,55	99,62 ± 0,54	99,89 ± 0,34

Forced degradation test *(Relative retention times of degradation product(s))*

	TRAMADOL	NEFOPAM
Wavelength	270 nm	225 nm
Acid hydrolysis	**0,79 et 4,99**	**0,65**
Oxidation	**1,18**	**1,16**

Basic hydrolysis & Oxidation: no degradation products observed under study conditions



PD TRA : Tramadol degradation product
PD NEF : Nefopam degradation product

CONCLUSION

The stability-indicating dosing method has been validated in accordance to the recommendations of the GERPAC[3] guide. The **development of a common method for the simultaneous assay of Tramadol and Nefopam**, for a stability study, is an asset, enabling us in particular to optimize handling time.

[1] *D'Huart. et al. Etude de stabilité physico-chimique d'une solution de néfopam et d'un mélange néfopam-dropéridol dilués en seringues polypropylène pour les services de soins intensifs. Hopipharm, 2019.*
[2] *Gu J. et al. Long term stability of Tramadol and Ketamine solutions for patient-controlled analgesia delivery. Med Sci Monit, 2015.*
[3] *SFPC et GERPAC. Guide méthodologique des études de stabilité des préparations. 1ère édition, avril 2013.*