Strategies for Correcting Peak Fronting of Oxycodone Hydrochloride, Naloxone Hydrochloride and Related Substances Observed in Reversed—Phase Liquid Chromatography

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PURPOSE

Oxycodone hydrochloride (HCl) and some of its impurities show peak fronting,[1] which prevents baseline separation from its related impurities and accurate peak area calculation. Peak fronting appears in many pharmaceuticals that can undergo keto-enol tautomerization. There is no published method for simultaneous determination of oxycodone HCl and naloxone HCl together in presence of their impurities.

OBJECTIVES

1. Develop and validate a reverse-phase ultra-performance liquid chromatography (RP-UPLC) assay for oxycodone HCl, naloxone HCl, and their impurities, oxycodone N-oxide, naloxone N-oxide, noroxymorphone and noroxycodone, in their extended-release tablets.
2. Investigate the use of ion pairing agents with different alkyl chain lengths (heptane, octane and nonane) to treat peak fronting.
3. Examine the role of mobile phase solvents in improving peak shape and resolution.

METHODS

UPLC System: **Waters® AcQuity with Waters® TUV detector, UV 210 nm.**

Gradient table

time (min.)	Buffer	Acetonitrile	Methanol
0	83.5	6.5	10
10	77	3	20
13	73	3	24
15	73	3	24
15.1	83.5	6.5	10

Buffer: Phosphate buffer pH 2.6 containing 0.05% 1-nonane sulfonate

Flow Rate: 0.85 mL/min. **Run Time**: 15 min.

Column: XBridge BEH C18, 2.5 □m, 2.1x100 mm.

Column Temperature: 40^{O}C.
Injection volume: 5 µL

Materials: USP oxycodone hydrochloride reference standard (RS),naloxone RS oxycodone N-oxide, naloxone N-oxide, noroxymorphone, sodium monohydrogen phosphate Phosphoric acid (85 %) Sodium1-heptane sulfonate, methanol and acetonitrile

METHODS (CONT.)

Validated method parameters include specificity, repeatability, intermediate precision, accuracy, linearity, range, limit of detection (LOD) and limit of quantitation (LOQ). Resolution and symmetry factor were calculated.

RESULTS

Method Development

- Acetonitrile provides better resolution, but the peak fronting increases, Figure 1A.
- Methanol is critical to obtain symmetric peaks. However, it does not provide enough resolution, Figure 1B.
- Optimal solvent system should contain the two solvents if resolution is critical
- Ion pairing agents improve peak shape and enhance resolutions. Longer side chain length, see Figure 2, 1-nonanesulfonate resulted in higher resolution than the other two ion pairing agents. The resolution between any of the critical impurities was more than 3.03 which means better selectivity for all impurities and robustness, Table 2.

Method Validation

- Selectivity: The resolution between any of the critical impurities was more than 3.03 which means better selectivity for all impurities. The symmetry factors were within the desired acceptable range between 0.95 and 1.1 for oxycodone and any of its related peaks, Figure 2.

Table 1: Summary of method validation results

Parameter	Naloxone HCl	Oxycodone HCl
Linearity: Range (mg/mL)	0.65 - 77.98	1.30 -156.05
Slope	26531.48	27050.02
Intercept	-30.85	-9234.78
R^2	0.9999	0.9999
LOD/LOQ (mg/mL)	0.087/0.26	0.032/0.097
injection repeatability RSD%	0.49%	0.44%
Repeatability (%RSD)	Day 1: 1.32% Day 2: 0.81% Day 3: 1.25%	Day 1: 1.33% Day 2: 1.29% Day 3: 0.79%
Intermediate Precision(%RSD):	2.21	3.09
Accuracy (% Recovery)	101.22	100.12

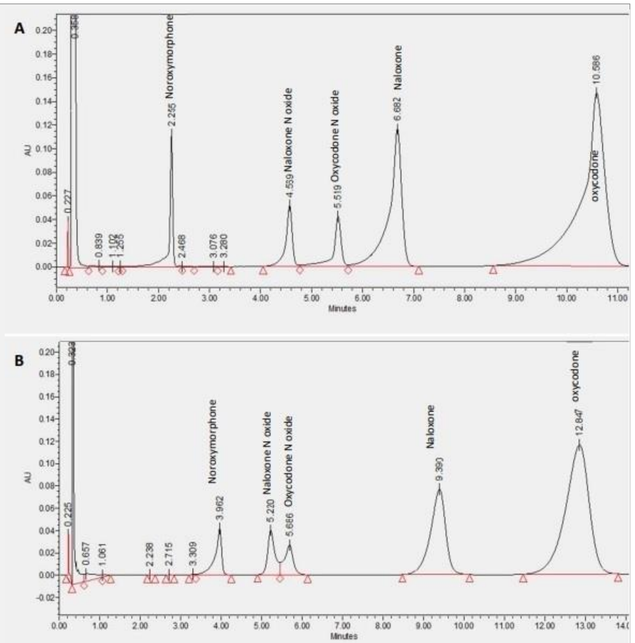

Figure 1: Use of methanol is associated with more symmetric peak shape as seen in chromatogram B but low resolution while use of acetonitrile is associated with better resolution as seen in chromatogram A but asymmetric peak shape.

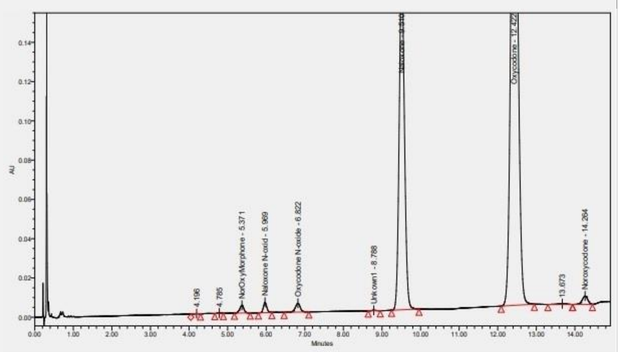

Figure 2: Example of chromatogram showing separation of naloxone N-oxide, oxycodone N-oxide, noroxymorphone and noroxycodone HCl at 1 µg/mL from naloxone HCl 70 µg/mL; oxycodone 140 µg/mL.

Table 2 Peak relative retention time, resolution and tailing parameters

Name	Relative Retention Time	USP Resolution	USP Tailing
NorOxyMorphone	0.437	3.03	0.92
Naloxone N-oxide	0.487	3.42	1.06
Oxycodone N-oxide	0.556	4.30	0.92
Unkown1	0.716	9.23	0.93
Naloxone	0.772	3.08	1.21
Oxycodone	1	9.58	1.29
Noroxycodone	1.1	3.42	1.11

CONCLUSIONS

An RP-UPLC method was developed and validated for separation of oxycodone HCl, naloxone HCl, and their critical impurities in extended-release tablets and in dissolution samples. Out of 3 ion pair agents tested, the best chromatographic resolution was obtained with 1-nonanesulfonate which has a longer side chain. Methanol is essential to correct peak fronting; however, it needs to be combined in a ternary organic solvent to improve resolution.

REFERENCE:

1. Broglé K, Ornaf RM, Wu D, Palermo PJ., J Pharm Biomed Anal. 1999 Apr;19(5):669-78.

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