Evaluation of Lidocaine Content and Delivery from Latex Elastrator Bands Using LC-MS, GC-MS and HPLC Techniques

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1. Introduction

Castration is a necessary management practice in agriculture. The health and safety of cattle and their handlers is improved via castration by reducing aggressiveness of bulls by lowering production of male hormones. Castration allows producers to prevent unwanted mating and produce meat has been shown to be consistently higher grade, with more marbling, allowing for beef to be sold at higher prices.[1] Helping manage the animal's pain during castration is important for the well-being of the animal and for a faster recovery time. The Canadian Code of Practice for the Care and Handling of Beef has recently been updated advising that pain control be used when bulls older than 6 months are castrated, and it is expected pain management will be advised for all castrations in the near future.[2] Lidocaine, a local anesthetic, is often injected during castration procedures to help mitigate pain; however it is relatively short acting and its injected delivery has many practical barriers. Recently, a novel way to administer lidocaine topically has been achieved by loading castration bands with the anaesthetic.[3] This strategy allows for continuous release of clinically significant doses of anesthetic to the animal upon application of the castration band, providing sustained pain relief. The Wulff lab has worked with Chinook Contract Research (CCR) in a NSERC Engage project to evaluate different sized castration bands loaded with lidocaine (shown below) using GC-MS, LC-MS and HPLC techniques. CCR carried out field studies to compare classical lidocaine injection with the novel castration band delivery method *in vivo*.

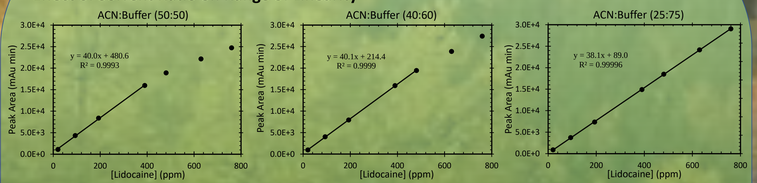
"Elastration Bander"

"Tri-Bander"

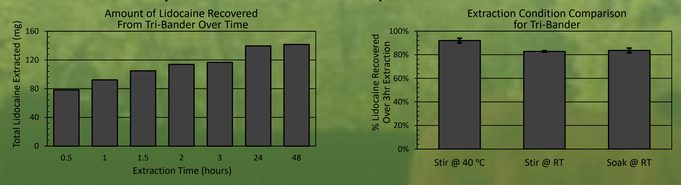
"Callicrate Bander"

2. HPLC Quantitation

Effect of Solvent Ratio on Range of Linearity

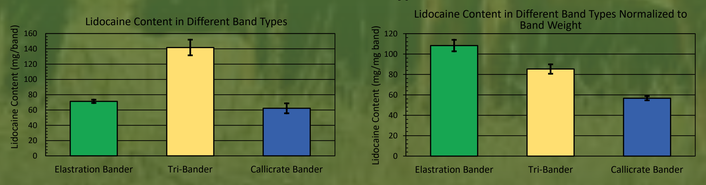


Band Extraction Example and Method Extraction Optimization



- The bulk of lidocaine (50-60% of total extract) is extracted in the first 30 minutes, indicating that over half of the lidocaine is loaded near the surface of the bands; this may be important for immediate pain relief.
- Different band extraction variables (such as solution agitation, heating and differing solvent amounts) were examined with no major improvements found compared to the simplest method, whereby the band is soaked in THF (50mL) at room temperature.

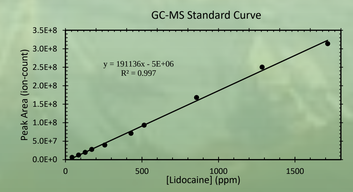
Lidocaine Content Determined in Different Band Types

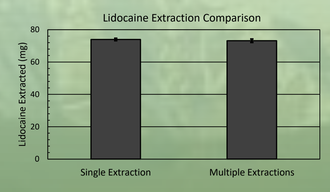


- All band types were shown to contain significant amounts of lidocaine capable of delivering therapeutic doses of lidocaine for pain relief.
- Standard deviation of the amount of lidocaine is small for each of the band types tested indicating manufacturing process is reliable and repeatable.

3. GC-MS Quantitation

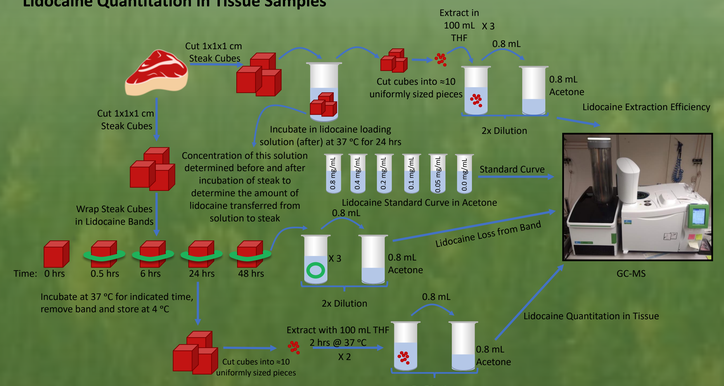
Lidocaine Quantitation in Elastrator Bands

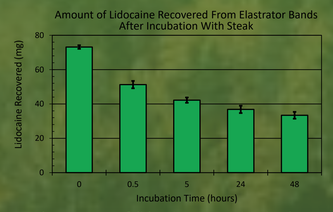


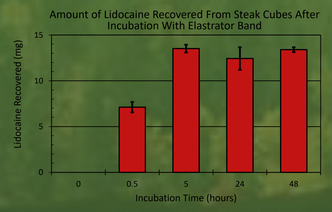


- GC (15 m x 0.25 mm, 0.5 µm stationary phase column) resulted in a wider linear range compared to HPLC.
- Extracting lidocaine once compared to multiple extractions (x3), over the same time period, resulted in the same amount of lidocaine recovered (73.93±0.71 mg/band and 73.16±1.04 mg/band respectively).

Lidocaine Quantitation in Tissue Samples



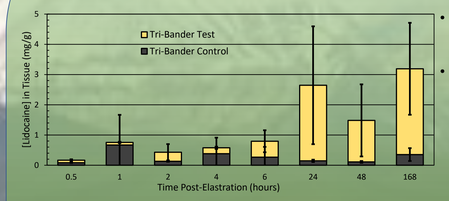




- The amount of lidocaine remaining in the castration bands (post-incubation with steak cubes) slowly decreases over time. The relatively large loss of lidocaine in the first 30 minutes suggests an initial high anesthetic release, followed by sustained slow release.
- The amount of lidocaine recovered from the steak cubes also showed this high initial uptake, however lidocaine recovery plateaued within the first five hours, indicating that a portion of the lidocaine being lost from the bands is unaccounted for.

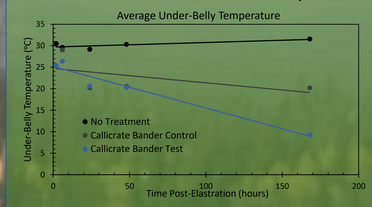
4. Field Study Results

Lidocaine Concentration in Bovine Scrotal Tissue

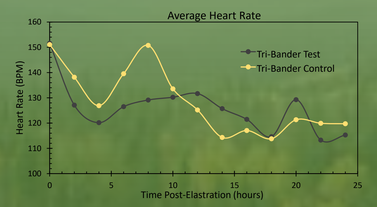


- 4 mm scrotal punch biopsies (skin and subcutaneous tissue) were taken over time for both test and control experimental groups.
- Small, and statistically insignificant, differences in lidocaine concentration were observed between the two groups for the first 6 hours, with lidocaine loaded Tri-Banders delivering higher, therapeutic levels of lidocaine over a longer time-scale.

Bovine Pain and Inflammation Response



- Under-belly temperature is positively correlated with inflammation.[4]
- The 2-fold decrease in temperature between the control and test groups after 7 days (20.2 °C and 9.27 °C respectively) suggests the Callicrate Bander decreased inflammation

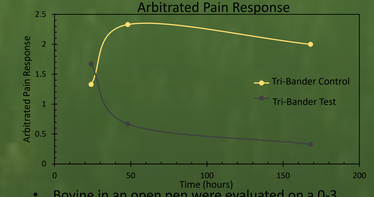


- Heart rate is positively correlated with animal stress.[4]
- Statistically significant data was not obtained to differentiate the two experimental groups based off heart rate.

Average Daily Gain

Animal Size at banding	Treatment Group (n=25/group)	ADG (Kg/Day)	±SD
>200 Kg	Control	0.52	0.42
	Test	0.80	0.55
>50 Kg	Control	0.91	0.59
	Test	0.94	0.44
<50 Kg	Control	0.73	0.24
	Test	0.89	0.65

- Average daily weight gain is an important indicator of animal health status.[4]
- While statistically significant data was not obtained to definitively distinguish the two groups, a weak trend was observed, motivating future larger experiments with a properly powered statistical study.



- Bovine in an open pen were evaluated on a 0-3 scale for various pain associated behaviours in an experimentally blind manner.
- The Tri-Bander test group was observed to exhibit decreased pain phenotypes over 7 days.

5. Conclusions & Future Directions

- Lidocaine content has been determined using a simplistic extraction method and quantified by HPLC and GC-MS instruments. Bands have been shown to contain significant levels of lidocaine.
- Initial *in vitro* and *in vivo* studies indicate the bands are capable of delivering lidocaine over extended periods of time which is advantageous over lidocaine administered by injection.
- Field studies are promising showing the lidocaine loaded bands work as expected eluting lidocaine into tissue. The efficacy of the bands was evaluated by physiological and behavioral techniques indicating the lidocaine loaded bands provide an ongoing numbing effect and pain relief compared to control bands with no lidocaine.
- Future research will help to support the eventual commercial scale up of this novel animal welfare product.

References

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Acknowledgements

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NSERC CRSNG