

DEVELOPMENT OF A MONOCLONAL ANTIBODY FOR THE DETECTION OF MEPERIDINE AND ITS METABOLITE NORMEPPERIDINE

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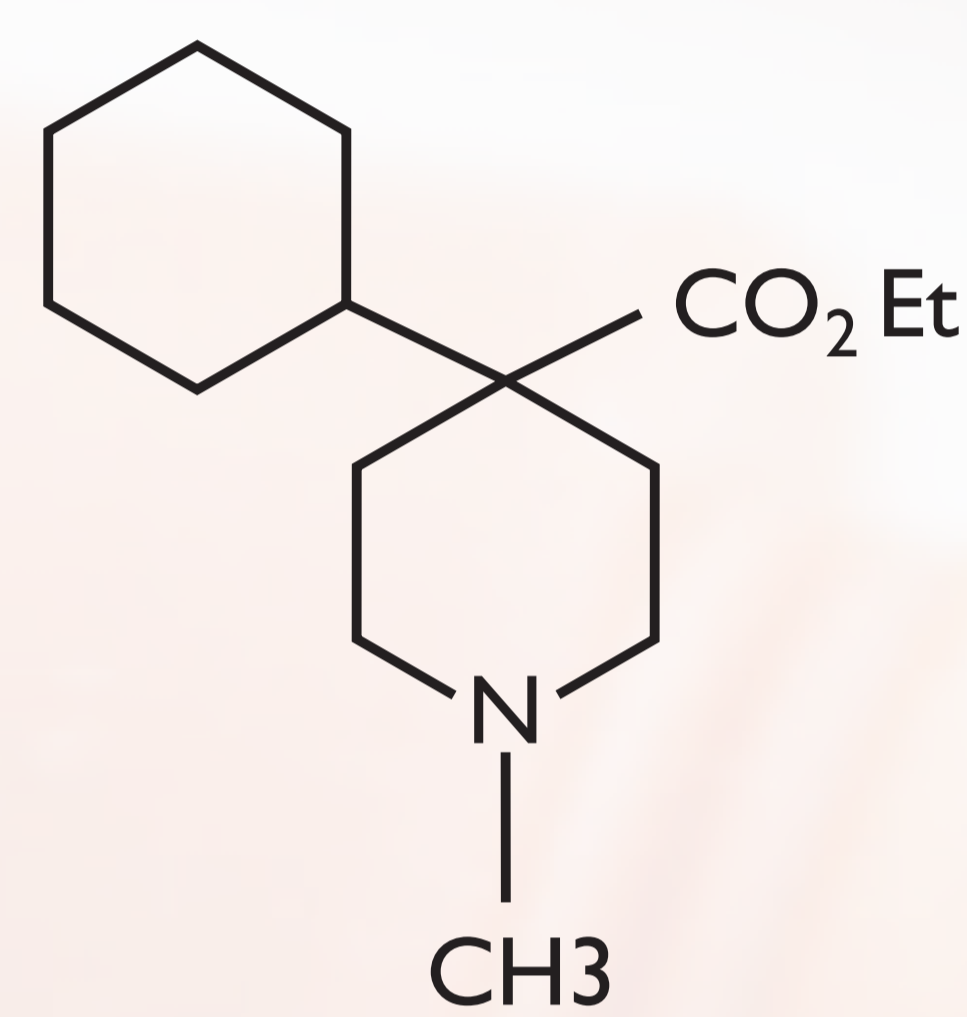
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Introduction

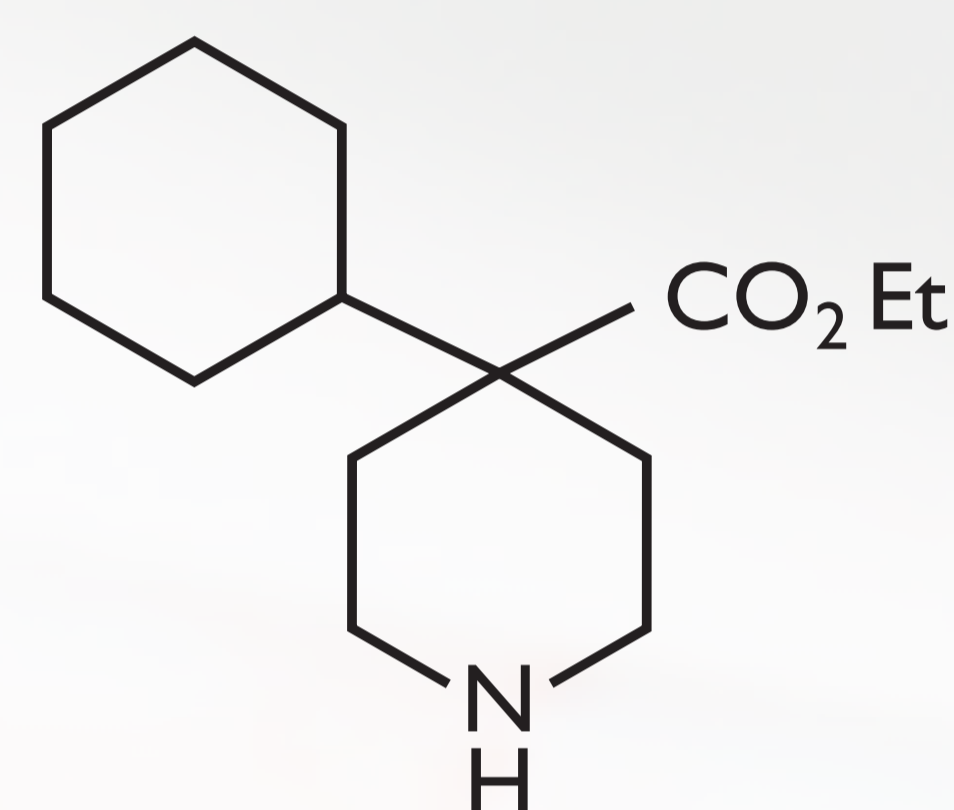
Meperidine was first introduced in the 1930s as an analgesic, producing effects that are similar to morphine.¹ It is currently used for pre-anaesthesia and the relief of moderate to severe pain. The metabolite normeperidine is approximately half as potent as meperidine, but it has twice the CNS stimulation effects, and has a longer half-life.¹ The Society of Forensic Toxicologists (SOFT) recommends meperidine and normeperidine as target analytes for meperidine.²

We report the development of a monoclonal antibody to meperidine and normeperidine, which is of value in developing more effective immunoassays for detecting these compounds and for applications to different settings. The antibody was immobilised on a biochip platform and the analytical parameters evaluation of the biochip-based immunoassay is reported.

Chemical structures



Meperidine



Normeperidine

Methodology

Sheep were immunized with normeperidine conjugated via an amino group to a carrier protein bovine thyroglobulin (BTG). Lymphocytes were collected and fused with heteromyeloma cells. The resulting hybridomas supernatants were screened for the presence of antibody using competitive ELISA based assays. Positive hybridomas were cloned to produce stable monoclonal hybridomas. The antibodies were purified and evaluated by competitive ELISA.

The optimal antibody was then immobilised on a biochip platform (9mm x 9mm), which is also the vessel for the immunoreactions. The semi-automated bench top analyser Evidence Investigator (EV3602, Radox Laboratories Ltd., Crumlin, UK) was used.

The assay principle is based on competition for binding sites of the monoclonal antibody between free antigen and horseradish peroxidase labelled conjugate. Sample and reagents are added to the biochip and incubated under controlled conditions. Following addition of substrate, a light signal is generated, which is then detected using digital imaging technology. The system incorporates dedicated software to automatically process, report and archive the data generated.

Assay evaluation parameters

Sensitivity: calibration curves were generated over 9 concentrations. The IC₅₀ was calculated by taking 50% of signal from the zero calibrator and reading the value from the x-axis (standard concentration in ng/ml) in the calibration curve. This corresponds to the concentration that produced 50% inhibition.

Specificity: specificity, expressed as % cross-reactivity (%CR) was calculated as follows:
 $\%CR = [IC_{50}(\text{analyte}) / IC_{50}(\text{cross-reactant})] \times 100$

Precision: intra-assay precision was determined from the results of replicates at different concentration levels within the same run. Results were expressed as %CV.

Results

Results corresponding to the initial antibody evaluation are presented.

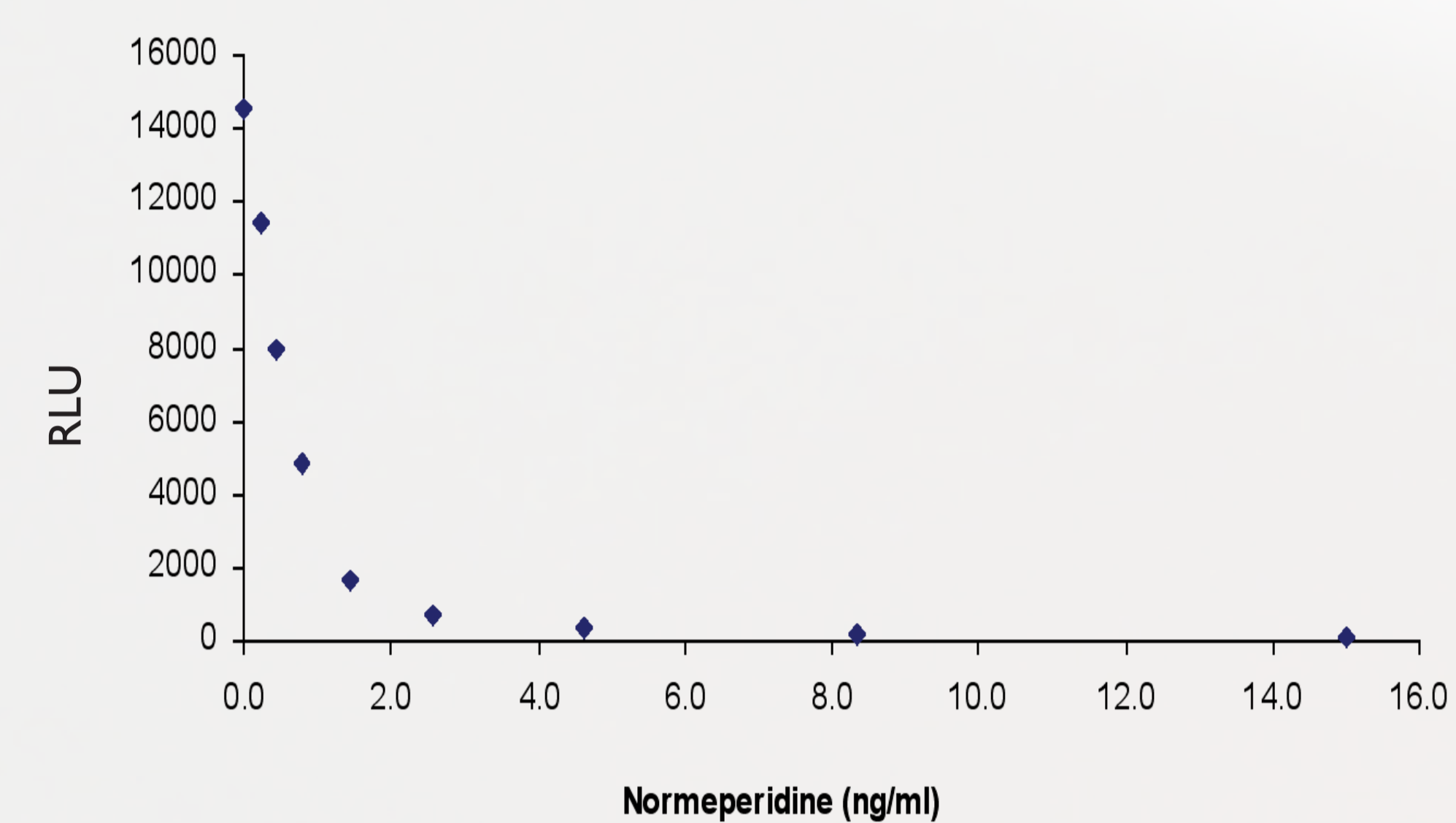
Sensitivity and specificity

Analyte	Calibration Range (ng/ml)	IC ₅₀ (ng/ml)
Normeperidine	0-15	0.491
Meperidine	0-15	0.479

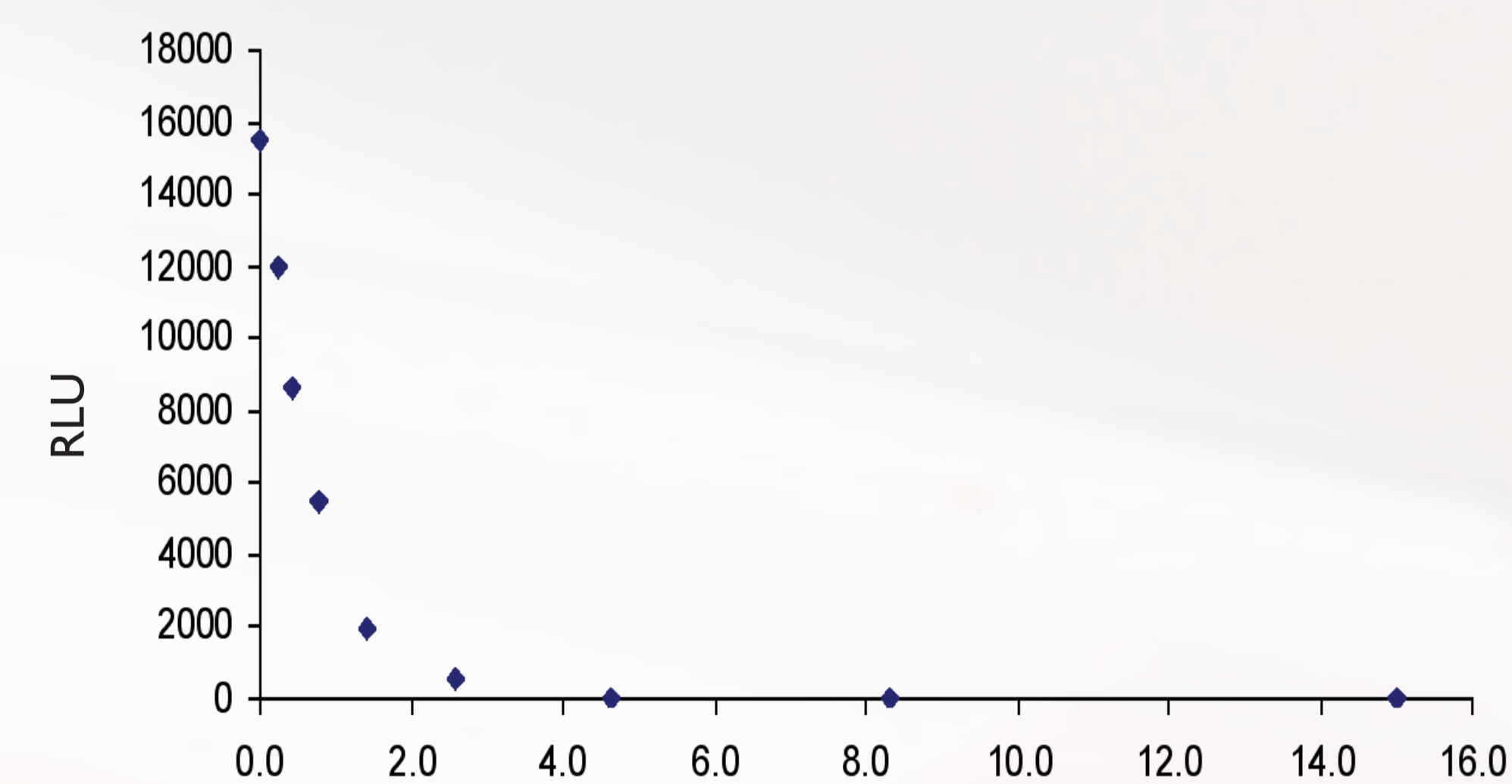
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Calibration curves

Normeperidine



Meperidine



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Meperidine (ng/ml)

Specificity

Zolpidem

Analyte	% Cross-reactivity
Normeperidine	100.0
Meperidine	102.5
Buprenorphine HCl	<1
Butorphanol tartrate	<1
Codeine	<1
Ethyl-glucuronide	<1
Fentanyl	<1
7-NH Flunitrazepam	<1
Hydrocodone	<1
Hydromorphone	<1
Levorphanol	<1
Morphine Sulfate Salt	<1
Meprobamate	<1
Methadone	<1
Nalbuphine	<1
Norketamine	<1
Oxycodone	<1
Oxymorphone	<1
Pentazocine	<1
Urochloralic acid	<1
Zaleplon	<1
Zolpidem	<1
Zopiclone	<1

11/164,169,208,215,217,248/459RDS

Typical intra-assay precision: %CV < 11 for different concentration levels.

Conclusion

Data indicate that the monoclonal antibody generated is suitable for the development of immunoassays for the determination of meperidine and normeperidine in test samples. The application to the biochip platform offers the option of multiplex screening using biochip array technology.

REFERENCES
 1. Baett R.C. Meperidine in Disposition of Toxic Drugs and Chemicals in Man, 8th edition, Biomedical Publications, Foster City, California, 2008, pp911-14.
 2. Society of Forensic Toxicologists (SOFT). 2005 Drug-Facilitated Sexual Assault Committee Recommended Maximum Detection Limits for Common DFSA Drugs and Metabolites in Urine Samples <http://www.soft-tox.org/files/SOFT-DFSA-List.pdf>