

HPLC/MS/MS Quantitative Bioanalysis Used in Homeland Security and Anti-Terrorism Efforts: Analysis of Atropine Used to Protect Against Chemical Warfare Agents

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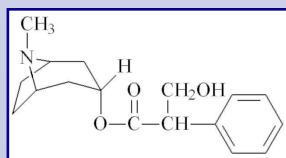
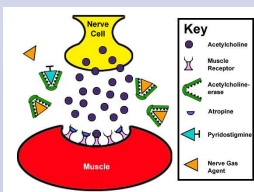
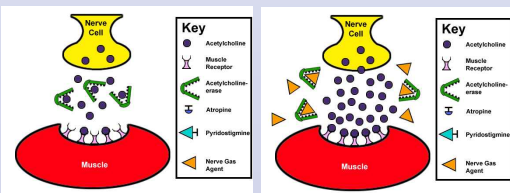
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Overview

- **Purpose** - Develop an HPLC/MS/MS method to determine low concentrations of atropine in monkey serum
- **Methods** – 96 well-plate extraction and HPLC/ESI/MS/MS (API3000)
- **Results** – Range from 0.01 to 10 ng/mL with accuracies and precision better than 20% using only 25 μ L of sample

Introduction

Atropine is an anticholinergic drug used to protect against chemical warfare nerve agents. New formulations and dosing regimens require an assay with quantitation limits near 10 pg/mL in monkey serum. To achieve a limit of quantitation of 10 pg/mL a recovery >65% for an HPLC/MS/MS assay will be required.



Atropine Structure

Methods

Extraction

- Precipitation of plasma proteins from 250 and 25 μ L of monkey serum in 96 well plates
- Investigate several methods of extraction
 - MTBE pH 11 and neutral
 - Acetonitrile
 - Diethyl ether
 - Dichloromethane
 - Solid phase extraction C18
 - Solid phase extraction polymer based
 - Brine/Acetonitrile extraction
- Extract, transfer aliquot, evaporate and reconstitute in 70 μ L of mobile phase

HPLC

- Gradient from 5% to 95% organic in 2 minutes.
- Flow rate = 0.6 mL/minute
- 2 mM ammonium formate, 0.1% formic acid mobile phase with MeOH
- Advantage Armor C8 (Analytical Sales and Service) 2.1 x 30 mm column
- Thirty μ L injections

Mass Spectrometry

- Sciex API3000 operating in MRM mode
- Turboionspray (400 °C)
- Positive ion mode
- MRM transitions –
 - β 290 → 124 (Atropine)
 - β 293 → 127 (D3 Internal Standard)

Extraction Challenges

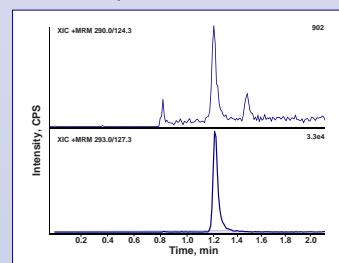
- Precipitation methods gave a non-selective extraction and recoveries <50%
- Solid-phase extraction methods on a “universal” polymer phase gave irreproducible recoveries
- Conventional liquid-liquid extractions gave recoveries <50%

Table 1. Development of an Extraction Method for the HPLC/MS/MS Analysis of Atropine from Monkey Serum

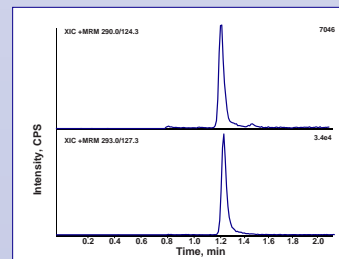
Extraction Procedure	% Recovery	% RSD
MTBE pH 11	32	13
MTBE	24	1.2
Dichloromethane	16	81
Acetonitrile	33	11
Diethyl Ether	40	60
SPE C18	32	20
SPE Polymer	76	40
Acetonitrile/Brine pH 11	72	8.8

Universal Liquid-Liquid Extraction for Biological Fluids

- Addition of acetonitrile with pH adjustment
- Addition of saturated NaCl solution
- Aqueous and acetonitrile layers separate
- Liquid-liquid extraction
- More selective, “cleaner extracts” than ACN precipitations
- Good recoveries for a wide-range of compounds



HPLC/MS/MS Chromatogram from the Analysis of a Standard Fortified with Atropine at 0.01 ng/mL and Extracted from Monkey Serum

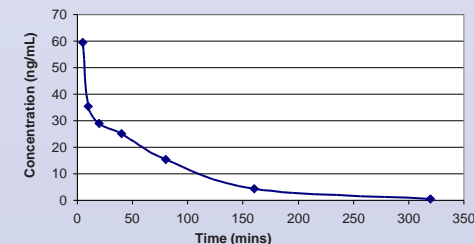


HPLC/MS/MS Chromatogram from the Analysis for Atropine of a Monkey Serum Sample 60 Minutes Post-Dose

Table 2. Standard Curve Results for the HPLC/MS/MS Analysis of Atropine from Monkey Serum.

Standard Curve Level (ng/mL)	Calculated Concentration (ng/mL)	% Accuracy
0.01	0.00943	94.3
0.01	0.0103	103
0.05	0.0492	98.5
0.05	0.0563	113
0.25	0.286	114
0.25	0.255	102
0.50	0.480	95.9
0.50	0.524	105
1.0	0.883	88.3
1.0	0.821	82.1
10	8.93	89.3

PK Curve for the Analysis of Atropine from Monkey Serum



Conclusions

- Developed HPLC/MS/MS method to quantify atropine from monkey serum
- Found that a liquid-liquid modified acetonitrile extraction gave best recoveries compared to other extractions
- Continue to support PK studies for new atropine formulations