Analysis of Atropine in Belladonna Extract (*Atropa Belladonna*) by HPLC

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Pharmaceutical

The tropane alkaloid atropine is the racemic form of hyoscyamine, which occurs in *Atropa belladonna*, *Datura stramonium*, *Hyoscyamus niger* and other extracts. The plant leaves contain about 0.3 - 0.7 % alkaloids. The very toxic compound was already used in the Middle Ages as a pharmaceutical drug, especially as a mydriatic. When dribbled into the eye, the belladonna extract causes the pupil to enlarge.

Figure 1 shows the separation of atropine in the extract of *Atropa belladonna* using gradient analysis on a reversed phase column and UV detection.

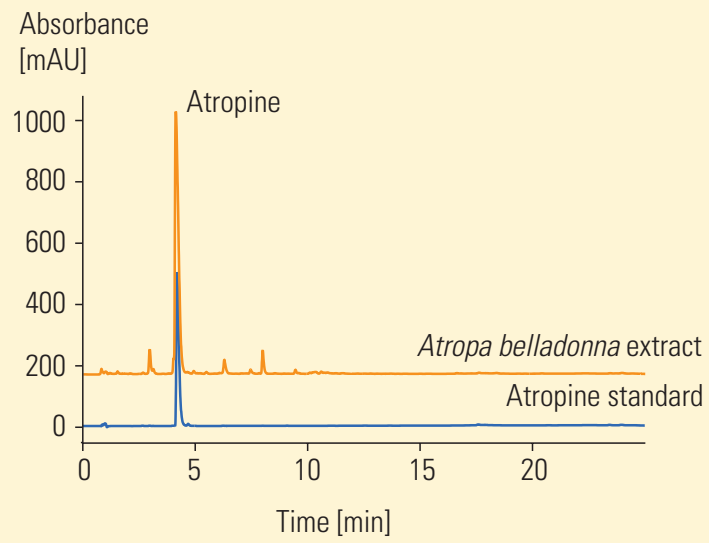

Figure 1
Analysis of *Atropa belladonna* extract

Conditions

Column
4.6 x 75 mm Zorbax Eclipse XDB-C18, 3.5 µm
Mobile phase
A = 0.05M KH_2PO_4 in water (pH = 3), B = acetonitrile
Flow rate
1.0 ml/min
Gradient
at 0 min 10 % B
at 20 min 60 % B
Column wash
at 23 min 60 % B
at 25 min 10 % B
UV detector
variable wavelength detector
210 nm, standard cell
Column compartment temperature
40 °C
Stop time
25 min
Post time 5 min
Injection volume 5 µl

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Extraction

1 g of the dried and powdered plant (from *Caesar & Loretz GmbH, Germany*) was refluxed for 30 min in 0.5 M acetic acid. After cooling the pH was adjusted to 9 and the solution was extracted five times with 50 ml chloroform. After drying over sodium sulfate the solvent was removed *i. vac.* and the residue dissolved in 2.5 ml methanol. After filtration 5 µl of the extract were applied to HPLC.

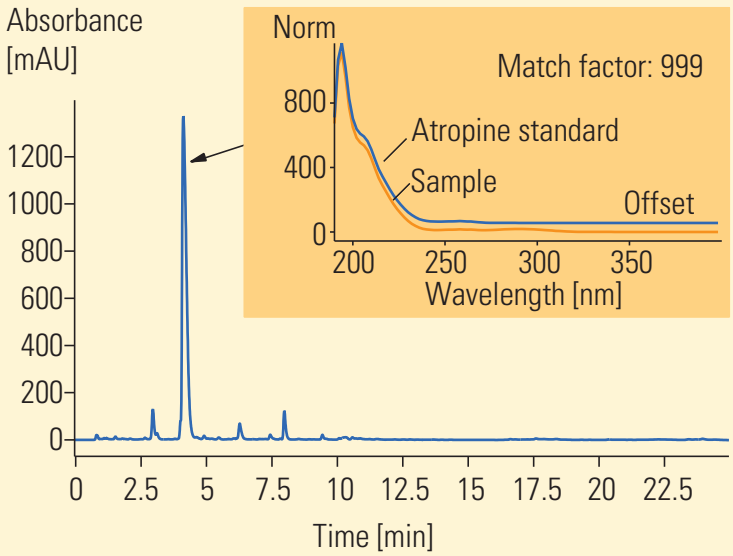

Figure 2
Comparison of sample and standard spectra of atropine

The method described here shows an easy but reliable and precise analysis of atropine in the extract of atropa belladonna.

Equipment

Agilent 1100 Series
- Quaternary pump (includes vacuum degasser
- Thermostatted autosampler
- Thermostatted column compartment
- Variable wavelength detector, standard flow cell 10-mm path length, 13-µl cell volume

Alternative:
- Binary pump
- Vacuum degasser
- Diode array detector standard flow cell 10-mm path length, 13-µl cell volume
- Agilent ChemStation + 3D software

Columns
- Zorbax Eclipse XDB C18, 3. 5 µm, 4.6 x 75 mm (Agilent part number 966967-902)
- *Recommended:* Guard cartridges Zorbax Eclipse XDB C18, 5 µm, 4 x 4 mm (Agilent part number 7995118-504, 10/pk)

Note:
Since the method was specifically developed on the Agilent 1100 Series system you might not be able to reproduce this analysis on an older system or even on a new system with lower performance.

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