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Quantitative Analysis of l-Hyoscyamine in *Hyoscyamus reticulatus* L. by GC-MS

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The hyoscyamine content of leaves and root samples of *Hyoscyamus reticulatus* L. from Bulanık, Muş in east Anatolia, Turkey, was determined by capillary GC-MS to be 0.036 ± 0.004% in the leaves and 0.056 ± 0.011% in the roots as the predominant compound. Sopolamine was only detected in trace amounts. These findings are in accordance with reports on hyoscyamine content in *Hyoscyamus* species. The limit of detection was 3.125 μg/mL and the limit of quantification was 6.25 μg/mL for hyoscyamine. This method has been shown to be linear and sensitive.

Key Words: l-Hyoscyamine, Hyoscyamus reticulatus L., Capillary GC-MS

Introduction

The routine quality control of a herbal drug preparation requires the identification and determination of the contents of the active ingredients in the product. Measurement alone is not sufficient; it is also necessary to know how objectively to evaluate the experimental results[1]. There are 6 *Hyoscyamus* (Solanaceae) species growing in Turkey, none of which are endemic[2]. *Hyoscyamus* (Solanaceae) species are rich sources of tropane alkaloids, mainly hyoscyamine and scopolamine, which are widely used for their mydriatic, antispasmodic, anticholinergic, analgesic and sedative properties[3,4]. Tropane alkaloids have been analyzed by many methods including aqueous and non-aqueous titrimetry, UV, visible spectrophotometry, TLC[5−7], gas chromatography (GC)[7−10], GC/MS[11], high performance liquid chromatography (HPLC)[8,12−18], capillary zone electrophoresis[19,20] and immunological methods[21]. The total alkaloid content of the *Hyoscyamus* species of Anatolia was determined by a colorimetric method to be 0.011-0.027% for the leaves and 0.417%, for the roots of *H. reticulatus*[22]. Abuse of *Hyoscyamus* by children and poisoning cases have been reported in Turkey[23,24]. The incidence of plant poisoning in Turkey is about 6% and is especially high among children

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between the ages of 2 and 11 living in rural areas[23]. *H. reticulatus* is used as a hallucinogenic drug in the east of Turkey. The objective of this study was to develop and validate a specific quality control method for l-hyoscyamine in herbal drug preparations.

Experimental

Material

H. reticulatus L. was collected from Bulanık, Muş (eastern Turkey) in May 2001 (AEF 22958).

Chemicals

l-Hyoscyamine (H-9002) was supplied by Sigma Chemicals.
Dichloromethane (M-106048), methanol (M-106002), petroleum ether (M- 100909) and chloroform (M-102447) wereobtained from Merck Chemicals.

Gas Chromatography

GC-MS was carried out on a Varian-Chrompack 3800 gas chromatograph coupled to a Saturn 2000 mass detector. A mass spectrometer with an ion trap detector in full scan (80-325 amu) under electron impact ionization (70 eV) was used. The chromatographic column for the analysis was a Chrompack WCOT-Fused Silica CP-Sil 5CB capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). The carrier gas used was helium at a flow rate of 1 mL/min. Then 1-μL crude alkaloid fractions were injected and analyzed with the column held initially at 125 °C for 1 min and then increased to 250 °C with a 10 °C/min heating ramp and subsequently kept at 250 °C for 5 min. The injection was performed in splitless mode at 280 °C.

All the calculations concerning the quantitative analysis were performed with external standardization by measurement of the peak areas.

Temperature Program

Temperature (°C)	Rate (°C/min)	Held (min)	Total Time (min)
125	—	1.00	1.00
125 - 250	10	12.5	13.5
250	—	5.00	18.5

Extraction of Alkaloids

Ten grams of dried and powdered roots and leaves of *H. reticulatus* L. were extracted with methanol in a Soxhlet apparatus for 2 h. The methanol was evaporated in vacuo at 50 °C to dryness. The pH of the residue was adjusted to 3 with 2 N HCl and then filtered. The filtrate was extracted 3 times with 20 mL of petroleum ether. The alkaloids were extracted 4 times with 50 mL of chloroform from the alkali aqueous solution (pH 10) using ammonia. The chloroform was evaporated to dryness and the residue was dissolved in 10 mL of dichloromethane. Then 1 μL of extract was directly injected into the gas chromatograph. The results were obtained as the mean of 3 separate injections.

Calibration

l-Hyoscyamine (12.00 mg) was accurately weighed into a 10 mL volumetric flask and dissolved and filled up to volume with dichloromethane. Standard solutions of l-hyoscyamine (6.250-1200 μg/mL) were prepared in the dichloromethane and 1 μL injections were performed in triplicate for each standard solution to see the reproducibility of the detector response at each concentration level. The peak area-ratio of each drug was plotted against the concentration to obtain the calibration graph. The 5 concentrations were subjected to regression analysis to calculate the calibration equation and correlation coefficients.

Results and Discussion

Linearity

Table 1 presents the equation of the regression line, determination coefficient, RSD values of the slope and intercept for l-hyoscyamine. Excellent linearity was obtained between peak-area ratios and concentrations of 6.250-1200 μg/mL with $r^2 = 0.9988$ for l-hyoscyamine.

Table 1. Linearity results, limit of detection (LOD) and limit of quantification (LOQ).

Compound	Equation	r^2	Slope (RSD %)	Intercept (RSD %)	LOQ μ g/mL	LOD μ g/mL
l-Hyoscyamine	Y= 120726886 X- 731526.6	0.9988	5.196	0.691	6.250	3.125

X = Concentration (μg/ mL); Y = Area

Limits of Detection and Quantification

Limits of detection (LOD) were established at a signal-to-noise ratio of 3. Limits of quantification (LOQ) were established at a signal-to-noise ratio of 9. LOD and LOQ were experimentally verified by 6 injections of l-hyoscyamine at the LOD and LOQ concentrations. The limit of detection was 3.125 μg/mL, and the limit of quantification was 6.250 μg/mL (Table 1).

Accuracy

Analysis of solution with a known concentration of l-hyoscyamine showed the accuracy of the method. A standard working solution containing 200 μg/mL was injected 6 times. The accuracy was expressed in terms of percentage deviation in the measured concentration (Table 2).

Table 2. Accuracy of the developed method (n = 6).

Compound	Spiked Concentration μg/mL	Measured Concentration μg/mL Mean ± SD	RSD %	Deviation %
l-Hyoscyamine	200	214.16 ± 14.58	6.81	7.08

$$\% \text{ Deviation} = \frac{\text{(Spiked Concentration - Mean Measured Concentration) x 100}}{\text{Spiked Concentration}}$$

Precision

The precision of the method (within-day variations of replicate determinations) was calculated by injecting l-hyoscyamine 5 times at the LOQ level. The precision of the method is expressed as the relative standard deviations (RSD %) at the LOQ level (Table 3).

Table 3. Precision of the developed method at the LOQ level (n = 5).

Compound	Peak Area (mean)	RSD %
l-Hyoscyamine	398728.8	7.56

Quantitative determination in *H. reticulatus* L.

Quantitative determination of l-hyoscyamine in the Bulanık samples was carried out by GC-MS using an external standard method. The area of peaks corresponding to standards were increased to prove the presence of these compounds (Table 4).

Table 4. Contents of l-hyoscyamine in *Hyoscyamus reticulatus* L.

l-hyoscyamine	Area (n = 3, mean)	Concentration μ g/mL (n = 3, mean)	Alkaloid % (n = 3, mean) Mean ± SD
Root	66983698.66	560.90	0.0561 ± 0.0011
Leaves	39030796.66	329.36	0.03294 ± 0.0059

Conclusion

Hyoscyamine, the predominant compound, reached 0.03294 ± 0.0059% in the leaves and 0.0561 ± 0.0011% in the roots. These findings are in accordance with reports on l-hyoscyamine content in *Hyoscyamus* species[25,26]. The limit of detection was 3.125 μg/mL and the limit of quantification was 6.25 μg/mL for l-hyoscyamine. Scopolamine was only detected in trace amounts.

Many GLC systems have been reported for the identification and quantitation of tropane alkaloids either in crude drugs or in pharmaceutical formulations. This system has been shown to be linear and sensitive. This accurate, simple and rapid method could be applied for the quality monitoring of l-hyoscyamine in biological materials.

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