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# NEW ANALYTICAL METHOD FOR THE MICRO-DETERMINATION OF MONOAMINE OXIDASE INHIBITOR DRUG SELEGILINE IN PHARMACEUTICAL FORMULATIONS AND URINE SAMPLES

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## ABSTRACT

*Ion-associate complexes of Selegiline hydrochloride; Sg HCl with [Co (II), Mn (II) thiocyanates], potassium ferricyanide and ammonium reineckate are precipitated. The solubility of the solid complexes at the recommended optimum conditions of pH and ionic strength values have been studied. Saturated solutions of each ion associate at different temperatures under the optimum precipitation conditions were prepared and the metal ion contents in the supernatant were determined. A new accurate and precise method based on inductively coupled plasma atomic emission spectrometry for the micro-determination of Selegiline hydrochloride (0.45-49.22 µg/ml) in pure solutions, pharmaceutical formulations and urine samples is given.*

***Keywords*** *– Selegiline hydrochloride, Pharmaceutical analysis, Ion-associate complexes, ICP-atomic emission spectrometry.*

## 1. INTRODUCTION

Selegiline hydrochloride (Sg) (Figure 1) (deprenyl), [(R)- (2)-N-methyl-(1-phenyl-2-propyl)-N-propinyl amine] hydrochloride [1], is a levo methamphetamine derivative which belongs to a class of drugs called phenethyl amines [2]. Sg is a selective, irreversible inhibitor of monoamine oxidase (MAO-A) [3]. It is used for the treatment of early stage Parkinson's disease, depression, and senile dementia [4]. It is useful adjunct in the treatment of cocaine addiction [5]. Sg (brand name Anipryl) is also used (at extremely high dosages relative to humans) in veterinary medicine to treat the symptoms of Cushing's disease and cognitive dysfunction (canine cognitive dysfunction) in dogs [6]. Recommended dosage of Sg is about 10mg/day; further increase in the dosage will lead to the nonselective inhibition of MAO [7]. Therefore, it is essential to develop a standard analytical method for monitoring residual drug in pure and in pharmaceutical formulations. Because of the pharmaceutical importance of drug has prompted us to devise methods for the rapid determination of Sg. So, we found it important to prepare new ion associates containing Sg and to study and elucidate their chemical structures to be applied to the analysis of Sg. A very few methods are reported in the literature for the determination of Sg in pharmaceutical formulations which include high performance liquid chromatography [8-12],gas chromatography [13-14], fluorescence polarization immunoassay and gas chromatography-mass spectrometry [15], spectro-

fluorometry [16], stereo selective analyses [17], reverse phase high performance liquid chromatography [18], spectrophotometry [19], flow injection chemiluminescence and corona discharge ion mobility spectrometry [20] and biosensor [21]. Though these methods are sensitive, they require expensive instruments, careful control of conditions, suffer from lack of selectivity, time consuming and trained personnel. Despite the availability of sophisticated and sensitive instruments, for routine quantitative analysis.

Although inductively coupled plasma atomic emission Spectrometry (ICP-AES) is a rapid method and has very low detection limits which cannot be reached by most of the above mentioned methods, it has not been applied yet to the determination of Sg HCl. The present work includes a new ICP-AES method for the micro-determination of Sg HCl. The method is based on precipitation of the ion associates formed from the reaction of Sg HCl with $[Co(SCN)_4]^{2-}$,$[Mn(SCN)_4]^{2-}$, $[Fe(CN)_6]^{3-}$ or ammonium reineckate; $[Cr(NH_3)_2(SCN)_4]^-$. The metal ion content present in saturated solutions of these ion associates is determined employing ICP-AES and is used to calculate the concentration of Sg HCl.

ICP-AES is well suited for this type of determination because of its accuracy, precision, sensitivity, and freedom from interference.

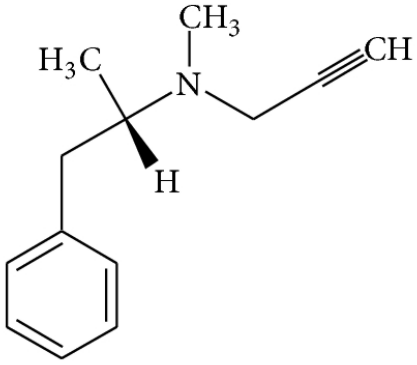


**Fig. 1: Chemical structure of Selegiline hydrochloride**

## 2. MATERIALS AND METHODS

Double-distilled water and analytical grade reagents were used to prepare all solutions. Selegiline hydrochloride ( Pfizer ), ammonium reineckate, potassium fericyanide, cobalt chloride and manganese chloride were Aldrich products, Selegiline Hexal, Selgin-5 and Eidepryl 5 Tablets, containing 5 mg Sg HCl per tablet were obtained from local pharmacy.

### 2.1 Apparatus

The pH of the solutions was measured using an Orion Research Model 701A digital pH-meter. Inductively coupled plasma atomic emission measurements were carried out using ICPE- 9000 Shimalspu plasma atomic emission spectrometer. Conductimetric measurements were carried out using conductivity measuring bridge type M.C.3 model EBB/10 ($K_{cell}$ = 1); [ Chertsey, Surry, England]. The IR absorption spectra were obtained by applying the KBr disk technique using a Pye Unicam SP – 300 infrared spectrometer.

### 2.2 Preparation of the Standard Solutions

The ion associates were prepared by mixing solutions containing 1 x $10^{-3}$ mol of Co (II), Mn (II) with a solution containing 4 x $10^{-3}$ mol of potassium thiocyanate and the requisite amount of Sg HCl. Potassium fericyanide and ammonium reineckate 1 x $10^{-3}$ mol of the solution was mixed with the calculated amount of Sg HCl. The precipitates obtained were filtered, thoroughly washed with distilled water, and dried at room temperature. They were subjected to elemental microanalysis, infrared spectroscopy, nuclear magnetic resonance, and determination of the metal content.

**2.3 Calibration of ICP-AES**

Under the recommended conditions, calibration graphs were constructed of aqueous standards of cobalt(II), manganese(II), iron(III) and chromium(III) in 1 M $HN0_3$ by performing triplicate measurements using solutions containing 0, 10, 20, and 50 ppm analyte concentrations as previously reported [22-23]. The calibration graphs are straight lines passing through the origin. The different parameters used for the measurement of cobalt(II), manganese(II), iorn(III) and chromium(III) are listed in Table 1.

**2.4 Conductimetric Measurements**

The stoichiometry of the ion associates was elucidated by conductimetric titration of Sg HCl with the metal complex solutions.

**2.5 Analytical Determination of Sg HCL in Aqueous Solutions**

Aliquots (0.05 - 5.5 ml) of 0.001 *M* Sg HCl solution are quantitatively transferred into 25-ml measuring flasks. To each flask 1.0 ml of 0.01 M standard solution of Co(II) and Mn(II) thiocyanate, fericyanide, or ammonium reineckate is added and the flask is filled to the mark with the recommended buffer solution of the optimum pH and ionic strength values. The solutions are shaken well and left to stand for 15 min and then filtered through Whatman P/S paper (12.5 cm), and the equilibrium metal ion concentration in the filtrate is determined using ICP-AES. The metal ion consumed in the formation of ion associates is calculated and the drug concentration is determined indirectly.

**2.6 Analytical Determination of Sg HCl in Pharmaceutical Formulations and Urine Samples**

The selegiline - containing pharmaceutical preparations (Selegiline Hexal, Selgin-5 and Eidepryl 5 Tablets) were successfully assayed using the present method. Sampling were made by grinding 20 Tablets then taking 1.75- 43.50, 2.25-42.75 and 2.50 - 41.25 µg / ml of the Selegiline Hexal, Selgin-5 and Eidepryl 5 Tablets, respectively. A certain amount of the cited drug within the applied concentration range (4.50 – 46.25 µg / ml) was spiked into urine of a healthy person. At the optimum conditions the tablets and urine samples were analyzed applying the above-mentioned procedure.

**3. RESULTS AND DISCUSSION**

**3.1 Composition and Structure of Ion-Associates**

The results of elemental analysis (Table 2) of the produced solid ion associates reveal that two duloxitinium cations form ion associates with one $[Co(SCN)_4]^{2-}$ or $[Mn(SCN)_4]^{2-}$ and three $[Fe(CN)_6]$,$^{3-}$ while only one Sg combines with $[Cr(NH_3)_2(SCN)_4]^-$ to form a 1:1 ion associate. These results are comparable to the previously reported results [22-25].

Conductimetric titrations of the investigated inorganic complexes with Sg HCl were performed to give insight into the stoichiometric compositions of the ion associates formed in solutions.

For all ion associates, the characteristic curves break at a molecular ratio ( [Sg ] / $[x]^{n-}$) of about 2, confirming the formation of 2:1 (Sg : $x^{2-}$) ion associates except in the case of the reineckate anion where the curve exhibits a sharp break at the 1:1 molecular ratio and in case of fericyanide anion the curve exhibits a sharp break at the 3 :1 molecular ratio . The results obtained coincide with the elemental analysis of the precipitated ion associate.

**3.2 Analytical Determination of sg HCl in Aqueous Solutions, Tablets, and Urine Samples**

Sg HCl was determined precisely and accurately in aqueous solutions, pharmaceutical formulations (Selegiline Hexal, Selgin-5 and Eidepryl 5 Tablets ) and urine using the present method. The results given in (Table 4 ) reveal that for ammonium reineckate the

recovery is 100.09 %, reflecting a high accuracy which in addition to the high precision indicated by very low values of relative standard deviations. For cobalt, manganese thiocyanate and fericyanide the recovery range is between 98.45 and 98.88 % -less accurate than that for ammonium reineckate.

**Table-1: Analytical Parameters for the Measurement of Co, Cr, Mn and Fe Using ICP- AES**

| Element | Wavelength (nm) | Order | Plasma position | DL (mg/L) | LDR (mg/L) | BEC (mg) | RSD x BEC (%) |
|---|---|---|---|---|---|---|---|
| Co | 236.37 | 95 | 0 | 0.02 | 0.2-1000 | 0.8 | 1 x 1.7 |
| Cr | 267.71 | 84 | 0 | 0.01 | 0.1-1000 | 0.4 | 7 x 0.7 |
| Mn | 257.61 | 87 | 0 | 0.003 | 0.03-100 | 0.1 | 1 x 0.7 |
| Fe | 248.30 | 90 | 0 | 0.01 | 0.1-1000 | 0.8 | 1 x 0.7 |

Note. DL, detection limit; LDR, linear dynamic range; BEC, background equivalent concentration; RSD, relative standard deviation. For all elements: state, ion; entrance slits, 50 x 300 µm; exit slits, 100 x 300 µm.

**Table-2: Elemental Analysis, Composition, and Some Physical Properties of Selegiline hydrochloride Ion - Associates.**

| Ion-associate composition | m. p.°C | Molar ratio | Color | % found (calculated) | | | |
|---|---|---|---|---|---|---|---|
| | | | | C | H | N | Metal |
| $(C_{13}H_{18}N)_2[Co(SCN)_4]$ | 382 | 2:1 | blue | 46.75 (46.79) | 5.34 (5.39) | 12.55(12.59) | 8.78 ( 8.85 ) |
| $(C_{13}H_{18}N)_2[Mn(SCN)_4]$ | 396 | 2:1 | white | 54.18 (54.29) | 5.36 (5.42) | 12.59 ( 12.66) | 8.24 ( 8.30) |
| $(C_{13}H_{18}N)$ $[Cr(NH_3)_2(SCN)_4]$ | 272 | 1:1 | violet | 40.26 (40.32) | 4.69 (4.74) | 19.31 (19.37) | 10.19 (10.28) |
| $(C_{13}H_{18}N)_3[Fe(CN)_6]$ | 355 | 3:1 | brown | 69.53 (69.58) | 6.89 (6.96) | 16.18 (16.24) | 7.17 (7.22) |

**Table-3: Solubility and Solubility Product Values at 25°C of Sg HCl Ion Associates at Their Optimum pH and Ionic Strength (µ) Values.**

| Ion Associate | pH | µ | pS | $pK_{sp}$ |
|---|---|---|---|---|
| $(Sg)_2[Co(SCN)_4]$ | 5.0 | 0.3 | 9.19 | 26.95 |
| $(Sg)_2[Mn(SCN)_4]$ | 6.0 | 0.5 | 9.33 | 27.39 |
| $(Sg)_3[Fe(CN)_6]$ | 4.0 | 0.6 | 8.47 | 32.43 |
| $(Sg)[Cr(NH_3)_2(SCN)_4]$ | 3.0 | 0.4 | 11.86 | 23.72 |

**Note:** pS, - log solubility, $pk_{sp}$, - log solubility product.

**TABLE 4: Analytical Determination of Selegiline hydrochloride in Aqueous Solution, Tablets and Urine Samples using ICP-AES**

| Sample | Amount taken (µg) | Mean recovery ( % ) | Mean RSD ( % ) |
|---|---|---|---|
| $[Co(SCN)_4]^{2-}$ | | | |
| Pure Sg HCl solution | 0.45 - 49.22 | 98.83 | 1.01 |
| Selegiline Hexal 5 | 1.75 - 43.50 | 98.88 | 1.03 |
| Selgin - 5 | 2.25 - 42.75 | 98.74 | 1.02 |
| Eidepryl 5 | 2.50 - 41.25 | 98.75 | 1.03 |
| Urine | 4.50 - 46.25 | 98.66 | 1.04 |
| $[Mn(SCN)_4]^{2-}$ | | | |
| Pure Sg HCl solution | 0.45 - 49.22 | 98.80 | 1.02 |
| Selegiline Hexal 5 | 1.75 - 43.50 | 98.79 | 1.01 |
| Selgin - 5 | 2.25 - 42.75 | 98.76 | 1.03 |
| Eidepryl 5 | 2.50 - 41.25 | 98.73 | 1.01 |
| Urine | 4.50 - 46.25 | 98.82 | 1.02 |
| $[Cr(NH_3)_2(SCN)_4]^{1-}$ | | | |
| Pure Sg HCl solution | 0.45 - 49.22 | 100.08 | 0.76 |
| Selegiline Hexal 5 | 1.75 - 43.50 | 100.05 | 0.74 |
| Selgin - 5 | 2.25 - 42.75 | 100.06 | 0.63 |
| Eidepryl 5 | 2.50 - 41.25 | 100.04 | 0.65 |
| Urine | 4.50 - 46.25 | 100.06 | 0.61 |
| $[Fe(CN)_6]3-$ | | | |
| Pure Sg HCl solution | 0.45 - 49.22 | 98.77 | 1.05 |
| Selegiline Hexal 5 | 1.75 - 43.50 | 98.74 | 1.03 |
| Selgin - 5 | 2.25 - 42.75 | 98.65 | 1.02 |
| Eidepryl 5 | 2.50 - 41.25 | 98.45 | 1.03 |
| Urine | 4.50 - 46.25 | 98.62 | 1.01 |

Note: RSD, relative standard deviation (six determinations)

## 4. CONCLUSION

The present method is applicable over a wider concentration range; (0.45-49.22 µg/ml) than that of Gupta and Paliwal [18] and Divya and Badiadka [19] where ( 80-130 ) and (10-85 and 20-120 ) µg/ml solution of Sg HCl can be determined, respectively.

In pharmaceutical analysis it is important to test the selectivity toward the excipients and the fillers added to the pharmaceutical preparations. Fortunately, such materials mostly do not interfere. This is clear from the results obtained for the pharmaceutical preparations (Table 4) that these excipients do not interfere.

Although the present method is more time consuming (20 min) in comparison to other methods such as (15 min for HPLC), it exhibits the advantages of simplicity, precision, higher sensitivity, accuracy, and convenience. Moreover, the reproducibility of the results are superior to those obtained from other methods such as gas chromatography [13-14]. Therefore, the method should be useful for routine analytical and quality control assay of the investigated drug in dosage forms.

In order to establish whether the proposed method exhibits any fixed or proportional bias, a simple linear regression [26] of observed drug concentration against the theoretical values (five points) was calculated. Student's t-test [27] (at 95% confidence level) was applied to slope of the regression line and showed that it didn't differ significantly from the ideal value of unity. Hence, it can be concluded that there are no systematic differences between the determination and true concentration over a wide range. The standard deviations (S.D.) can be considered satisfactory at least for the level of concentrations examined.

Although the present method is more time consuming than some other methods, it exhibits fair sensitivity and accuracy. Moreover, the reproducibility of the results is superior to that obtained from other methods

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