QUANTITATIVE ANALYSIS OF WARFARIN SODIUM AMORPHOUS IN ORAL SUSPENSION USING RAMAN SPECTROSCOPY

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Active Pharmaceutical Ingredient (API) Warfarin Sodium, used as an anticoagulant, can be found in three formulations: as a tablet, oral suspension (syrup) with strength of 1 mg/mL and dilution for injections. Warfarin Sodium is the sodium salt of 3-(α-acetonylbenzyl)-4-hydroxycoumarin, a compound which is available in two solid forms, amorphous and crystalline clathrate. The crystalline clathrate form is warfarin sodium-isopropyl alcohol complex [1]. In the present study a method of quantitative analysis of the API in the syrup was developed, using Raman Spectroscopy. Raman spectroscopy is vibration technique that, as oppose to XRPD, can identify the presence of an amorphous material and to differentiate it from another polymorph state. Also it can be successfully used for quantitative determination being much less cumbersome and time consuming than the suggested from the Pharmacopoeia HPLC method.

The spectrum of solid form of the amorphous Warfarin Sodium added in the suspension exhibits a single strong peak at 1606 cm^{-1} (Fig. 1; red line). After its dispersion in placebo, being mostly a mixture of water propylene glycol and liquid maltitol along with other solid excipients, two API peaks at 1573 and 1612 cm^{-1} appear. The presence of the two peaks indicates a possible transformation of the amorphous Warfarin Sodium to another state. For the quantitative analysis the ratios of two vibration peaks' areas of API, to the area of a strong excipient peak at 923 cm^{-1}, were calculated.

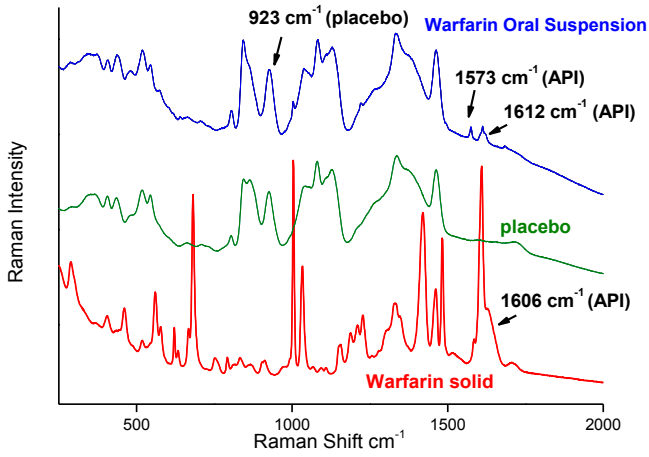

Fig. 1. Raman spectra of Warfarin Oral Suspension, placebo and Warfarin Sodium amorfous

For constructing the calibration line five dispersions were used as standards. The first four were made by adding placebo to commercially available syrup (25% syrup-75% placebo, 50% syrup- 50% placebo, 75% syrup- 25% placebo and 100% syrup) and the last one by enriching placebo with solid API in order to achieve a concentration 120% compared to the suspension's concentration. Samples were placed on a glass slide coated with a gold mirror-like substrate and were dried in the oven for 30 minutes at 100 °C in order to obtain a more concentrated, as wt %, gel suspension as the major liquid constituents of the suspension (water and propylene glycol) were evaporated.

Slides were placed on a home-made rotating apparatus (photo 1). In this way it was possible to collect the Raman signal from the circumference of a circle formed during sample's rotation, minimizing the under-sampling problems.

Photo 1. Home-made rotating apparatus for minimizing the under-sampling problems

Initial results indicate that there is a linear correlation between the concentration of API and Raman's signal (ratio of vibration peaks' areas of API to the area of the strong excipients peak respectively). Calibration line was also found to depend on the days that the samples remain on the slide before the measurements, due to the continuous evaporation of an excipient.

References

[1]. Gao D, Maurin MB. "Physical chemical stability of warfarin sodium". The AAPS Journal; 3 (2001) 18-25. (DOI: 10.1208/ps030103)